Hines J.
09/272809

FILE 'CAPLUS' ENTERED AT 14:11:03 ON 13 SEP 1999
L1 6668 S APOPROTEIN OR APO PROTEIN
L2 14 S L1 AND SYNECHOCYST?
L3 0 S L1 AND (CPH2 OR CPH 2)

- key terms

=> d l2 1-14 .beverly

L2 ANSWER 1 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:387378 CAPLUS
TITLE: Extended Heme Promiscuity in the Cyanobacterial Cytochrome c Oxidase: Characterization of Native Complexes Containing Hemes A, O, and D, Respectively
AUTHOR(S): Fromwald, Susanne; Zoder, Roland; Wastyn, Marnik; Lubben, Mathias; Peschek, Gunter A.
CORPORATE SOURCE: Molecular Bioenergetics Group, Institute of Physical Chemistry, University of Vienna, Vienna, A-1090, Austria
SOURCE: Arch. Biochem. Biophys. (1999), 367(1), 122-128
CODEN: ABBIA4; ISSN: 0003-9861
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The cyanobacteria Anacystis nidulans (Synechococcus sp. PCC6301), **Synechocystis** sp. PCC6803, Anabaena sp. PCC 7120, and Nostoc sp. PCC8009 were grown photoautotrophically under reduced oxygen tension in a medium with sulfate replaced by thiosulfate and nitrate replaced by ammonium as the S- and N-sources, resp. In addn., Anabaena and Nostoc were grown under dinitrogen-fixing conditions in a medium free of combined nitrogen. Membranes were isolated from late-logarithmic cells (culture d. corresponding to approx. 3 .mu.l packed cells per mL); cytoplasmic and thylakoid membranes were sepd. and purified according to established procedures. Acid-labile hemes were extd. from the membranes and subjected to reversed-phase high-performance liq. chromatog. Sepd. hemes were analyzed spectroscopically and identified by comparison with authentic stds. In addn. to hemes B, A, and O, the latter of which was induced under semianaerobic conditions only, substitution of thiosulfate and ammonium for the oxy-anions sulfate and nitrate led to the appearance of spectrally discernible heme D in the membranes and exts. therefrom. However, spectroscopic and kinetic investigation of the membrane-bound heme D rather disproved any reaction with oxygen or carbon monoxide. Kinetic measurements performed with the membrane-bound respiratory oxidase gave evidence for only two kinetically competent terminal oxidases, a3 and o3, both apparently assocd. with a single type of **apoprotein**, viz. subunit I of the known cyanobacterial aa3-type cytochrome c oxidase. The heme D, on the other hand, seems to form a spectrally distinguished, yet kinetically ill-defined hemoprotein complex which

does not qualify as a fully functional d-type terminal oxidase on our (wild-type) cyanobacteria even after growth under semianaerobic pseudo-reducing conditions. Also growth (of Anabaena and Nostoc) under dinitrogen-fixing conditions did not change this situation. Thus, we are left with (wild-type) cyanobacteria forming an unbranched respiratory chain with only a single type of terminal oxidase protein, viz. the known aa3-type cytochrome c oxidase. This oxidase, however, may incorporate different prosthetic (heme) groups in the sense of "heme promiscuity. " Biosynthesis of the different heme groups thereby seems to respond to the ambient redox environment. In particular, however, conditions for expression of the two quinol oxidases potentially and addnl. coded for by the genome of, e.g., **Synechocystis** sp. PCC6803 (see), have not yet been found. (c) 1999 Academic Press.

L2 ANSWER 2 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:753354 CAPLUS
DOCUMENT NUMBER: 130:22819
TITLE: The system of phytochromes: photobiophysics and photobiochemistry in vivo
AUTHOR(S): Sineshchekov, V. A.
CORPORATE SOURCE: Department of Biology, Lomonosov Moscow State University, Moscow, 119899, Russia
SOURCE: Biol. Membr. (1998), 15(5), 549-572
CODEN: BIMEE9; ISSN: 0233-4755
PUBLISHER: Nauka
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Russian

AB A review with 110 refs. The major achievement in the recent investigations of phytochrome, a key photoregulation pigment in plants, is the discovery of its structural and functional heterogeneity. A small family of phytochromes which differ by the **apoprotein** was detected by means of immunochem., mol. biol. and genetics. We have been approaching this problem by investigating the chromophore component of the pigment by means of the developed method of in-vivo low-temp. fluorescence spectroscopy of phytochrome. In etiolated plants, phytochrome fluorescence was detected and attributed to its red-light absorbing form (Pr) and the first photoproduct (lumi-R). A scheme of the photoreaction in phytochrome, a distinction of which is the activation barrier in the excited state, was put forward. The spectroscopic and photochem. characteristics of Pr were found to depend on the plant species and phytochrome mutants and overexpressors used, on localization of the pigment in organs and tissues, plant age, effect of preillumination and other physiol. factors. This variability of the parameters was interpreted as the existence of at least two phenomenol. Pr populations, which differ by their spectroscopic characteristics and activation parameters of the Pr .fwdarw. lumi-R photoreaction. Phytochrome B (phyB) accounts for less than 10% of the total

phytochrome fluorescence. It belongs to the Pr" type and is also characterized by a relatively low photoconversion into the far-red-light absorbing physiol. active phytochrome form, Pfr. Fluorescence of the minor phytochromes (phyC-phyE) is negligible. The recently discovered phytochrome of the cyanobacterium **Synechocystis** also belongs to the phenomenol. Pr" type PhyA' is a light-labile and sol. fraction, while phyA" is a relatively light-stable and, possibly, membrane (protein)-assocd. Expts. with transgenic tobacco plants overexpressing full-length and C- and N-terminally truncated oat phytochrome A suggest that phyA' and phyA" differ by the post-translational modification of the small N-terminal segment (amino acid residues 7-69) of the pigment. PhyA' is likely to be active in the de-etiolation processes while phyA", together with phyB, in green plants as revealed by the expts. on transgenic potato plants and phytochrome mutants of Arabidopsis and pea with altered levels of phytochromes A and B and modified phenotypes. And finally, within phyA', there are three subpopulations which are, possibly, different conformers of the chromophore. Thus, there is a hierarchical system of phytochromes which include: (1) different phytochromes: (2) their post-translationally modified states and (3) conformers within one mol. type. Its existence might be the rationale for the multiplicity of the photoregulation reactions in plants mediated by phytochrome.

L2 ANSWER 3 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:122457 CAPLUS
DOCUMENT NUMBER: 128:279901
TITLE: Fluorescence and photochemistry of recombinant phytochrome from the cyanobacterium **Synechocystis**
AUTHOR(S): Sineshchekov, V.; Hughes, J.; Hartmann, E.; Lamparter, T.
CORPORATE SOURCE: Biology Department, M. V. Lomonosov Moscow State University, Moscow, 119899, Russia
SOURCE: Photochem. Photobiol. (1998), 67(2), 263-267
CODEN: PHCBAP; ISSN: 0031-8655
PUBLISHER: American Society for Photobiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Fluorescence and photochem. properties of phytochrome from the cyanobacterium **Synechocystis** were investigated in the temp. interval from 293 to 85 K. The **apoprotein** was obtained by overexpression in Escherichia coli and assembled to a holo-phytochrome with phycocyanobilin (PCB) and phytochromobilin (P.PHI.B), Syn(PCB)phy and Syn(P.PHI.B)phy, resp. Its red-absorbing form, Pr, is characterized at 85 K by the emission and excitation maxima at 682 and 666 nm in Syn(PCB)phy and at 690 and 674 nm in Syn(P.PHI.B)phy. At room temp., the spectra are blue shifted by

5-10 nm. The fluorescence intensity dropped down by .apprx.15-20-fold upon warming from 85 to 293 K and activation energy of the fluorescence decay was estd. to be ca 5.4 and 4.9 kJ mol-1 in Syn(PCB)phy and Syn(P.PHI.B)phy, resp. Phototransformation of Pr upon red illumination was obsd. at temps. above 160-170 K in Syn(PCB)phy and above 140-150 K in Syn(P.PHI.B)phy with a 2-3 nm shift of the emission spectrum of the blue and increase of the intensity of its shorter wavelength part. This was interpreted as a possible formation of the photoproduct of the meta-Ra type of the plant phytochrome. At ambient temps., the extent of the Pr phototransformation to the far-red-absorbing form, Pfr, was ca 0.7-0.75 and 0.85-0.9 for Syn(PCB)phy and Syn(P.PHI.B)phy, resp. Fluorescence of Pfr and of the photoproduct similar to lumi-R was not obsd. With respect to the photochem. parameters, Syn(PCB)phy and Syn(P.PHI.B)phy are similar to each other and also to a small fraction of phyA (phyA") and to phyB. The latter were shown to have low photochem. activity at low temps. in contrast to the major phyA pool (phyA'), which is distinguished by the high extend (ca 50%) of Pr phototransformation at 85 K. These photochem. features are interpreted in terms of different activation barriers for the photoreaction in the Pr excited state.

L2 ANSWER 4 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:772086 CAPLUS
DOCUMENT NUMBER: 128:112486
TITLE: The phytofluors: a new class of fluorescent protein probes
AUTHOR(S): Murphy, John T.; Lagarias, J. Clark
CORPORATE SOURCE: Section of Molecular and Cellular Biology, University of California, Davis, CA, 9561 6, USA
SOURCE: Curr. Biol. (1997), 7(11), 870-876
CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER: Current Biology Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Biol. compatible fluorescent protein probes, particularly the self-assembling green fluorescent protein (GFP) from the jellyfish Aequorea victoria, have revolutionized research in cell, mol. and developmental biol. because they allow visualization of biochem. events in living cells. Addnl. fluorescent proteins that could be reconstituted in vivo while extending the useful wavelength range towards the orange and red regions of the light spectrum would increase the range of applications currently available with fluorescent protein probes. Intensely orange fluorescent adducts, which we designate phytofluors, are spontaneously formed upon incubation of recombinant plant phytochrome **apoproteins** with phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin chromophore. Phytofluors have large molar absorption coeffs., fluorescence quantum yields greater than 0.7, excellent

photostability, stability over a wide range of pH, and can be reconstituted in living plant cells. The phytofluors constitute a new class of fluorophore that can potentially be produced upon bilin uptake by any living cell expressing an apophytochrome cDNA. Mutagenesis of the phytochrome **apoprotein** and/or alteration of the linear tetrapyrrole precursor by chem. synthesis are expected to afford new phytofluors with fluorescence excitation and emission spectra spanning the visible to near-IR light spectrum.

L2 ANSWER 5 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:751576 CAPLUS
DOCUMENT NUMBER: 128:84943
TITLE: Characterization of a gene encoding dihydrolipoamide dehydrogenase of the cyanobacterium **Synechocystis** sp. strain PCC 6803
AUTHOR(S): Engels, Anke; Pistorius, Elfriede K.
CORPORATE SOURCE: Universitat Bielefeld, Biologie VIII: Zellphysiologie, Bielefeld, 33501, Germany
SOURCE: Microbiology (Reading, U. K.) (1997), 143(11), 3543-3553
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The authors previously reported the isolation and partial characterization of a periplasmically located dihydrolipoamide dehydrogenase (LPD) from the cyanobacterium **Synechocystis** sp. strain PCC 6803. In the present work the gene (lpdA; database accession no. Z48564) encoding the **apoprotein** of this LPD in **Synechocystis** PCC 6803 has been identified, sequenced and analyzed. The lpdA gene codes for a protein starting with methionine, which is post-translationally removed. The mature protein contains an N-terminal serine and consists of 473 amino acids with a deduced mol. mass of 51421 Da (including one FAD). The LPD is an acidic protein with a calcd. isoelec. point of 5.17. Comparison of the amino acid sequence of the **Synechocystis** LPD with protein sequences in the databases revealed that the enzyme shares identities of 31-35% with all 18 LPDs so far sequenced and published. As a first step in detg. the role of this cyanobacterial LPD, attempts were made to generate an LPD-free **Synechocystis** mutant by insertionally inactivating the lpdA gene with a kanamycin-resistance cassette. However, the selected transformants appeared to be heteroallelic, contg. both the intact lpdA gene and the lpdA gene inactivated by the drug-resistance cassette. The heteroallelic mutant studied, which had about 50% of the wild-type LPD activity, caused acidification of the growth medium. Growth over a prolonged time was only possible after an increased buffering of the medium. Since it is reported in the

literature that inactivation of the pyruvate dehydrogenase complex (PDC) leads to acidosis, a function of the LPD in a cytoplasmic-membrane-assocd. PDC is conceivable.

L2 ANSWER 6 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:710493 CAPLUS
DOCUMENT NUMBER: 128:31606
TITLE: Characterization of recombinant phytochrome from the cyanobacterium **Synechocystis**
AUTHOR(S): Lamparter, Tilman; Mittmann, Franz; Gartner, Wolfgang; Borner, Thomas; Hartmann, Elmar; Hughes, Jon
CORPORATE SOURCE: Institut fur Pflanzenphysiologie und Mikrobiologie, Freie Universitat, Berlin, D-14195, Germany
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1997), 94(22), 11792-11797
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The complete sequence of the **Synechocystis** chromosome has revealed a phytochrome-like sequence that yielded an authentic phytochrome when overexpressed in Escherichia coli. In this paper we describe this recombinant **Synechocystis** phytochrome in more detail. Islands of strong similarity to plant phytochromes were found throughout the cyanobacterial sequence whereas C-terminal homologies identify it as a likely sensory histidine kinase, a family to which plant phytochromes are related. An .apprxeq.300 residue portion that is important for plant phytochrome function is missing from the **Synechocystis** sequence, immediately in front of the putative kinase region. The recombinant **apoprotein** is sol. and can easily be purified to homogeneity by affinity chromatog. Phycocyanobilin and similar tetrapyrroles are covalently attached within seconds, an autocatalytic process followed by slow conformational changes culminating in red-absorbing phytochrome formation. Spectral absorbance characteristics are remarkably similar to those of plant phytochromes, although the conformation of the chromophore is likely to be more helical in the **Synechocystis** phytochrome. According to size-exclusion chromatog. the native recombinant **apoproteins** and holoproteins elute predominantly as 115- and 170-kDa species, resp. Both tend to form dimers in vitro and aggregate under low salt conditions. Nevertheless, the purity and soly. of the recombinant gene product make it a most attractive model for mol. studies of phytochrome, including x-ray crystallog.

L2 ANSWER 7 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:277197 CAPLUS

DOCUMENT NUMBER: 126:340775
TITLE: A prokaryotic phytochrome
AUTHOR(S): Hughes, Jon; Lamparter, Tilman; Mittmann, Franz; Hartmann, Elmar; Wilde, Annegret; Borner, Thomas
CORPORATE SOURCE: Institut fur Pflazenphysiologie und Mikrobiologie, Freie Universitat Berlin, Berlin, D-14195, Germany
SOURCE: Nature (London) (1997), 386(6626), 663
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Macmillan Magazines
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A recently described sequence of **Synechocystis** appeared to encode a phytochrome-like protein (Kaneko, T. et al., 1996). Translation of the **Synechocystis** PCC 6803 ORF slr0473 (the putative phy gene) yielded a product similar to plant phytochromes and, in the C-terminal domain, to bacterial sensory kinases. The putative phy gene product was further investigated by expression-cloning in Escherichia coli. Expression of the PHY **apoprotein** was very efficient. Recombinant His-tagged product, purified by affinity chromatog., autocatalytically attached phycocyanobilin (PBC). The spectral properties of the holoprotein were reminiscent of plant phytochrome-PBC adducts with absorbance maxima at 658 and 702 nm after red and far-red irradn., resp., and an isobestic point at 677 nm. This is the 1st report of a spectrally functional prokaryotic phytochrome.

L2 ANSWER 8 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1996:337789 CAPLUS
DOCUMENT NUMBER: 125:29017
TITLE: Promiscuity of heme groups in the cyanobacterial cytochrome-c oxidase
AUTHOR(S): Auer, Gudrun; Mayer, Bernhard; Wastyn, Marnik; Fromwald, Susanne; Eghbalzad, Khosrow; Alge, Daniel; Peschek, Guenter A.
CORPORATE SOURCE: Institute of Physical Chemistry, University of Vienna, Vienna, A-1090, Austria
SOURCE: Biochem. Mol. Biol. Int. (1995), 37(6), 1173-1185
CODEN: BMBIES; ISSN: 1039-9712
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The cyanobacteria Nostoc sp. strain Mac, Anabaena 7937, **Synechocystis** 6803, and Anacystis nidulans (Synechococcus 6301) were grown and incubated in the light under three different oxygen regimes: Phase-A cells were harvested from photoautotrophically growing cultures at a cell d. of 2.8-3.2 .mu.L packed cell mass/mL and an oxygen concn. of approx. 350 .mu.M (corresponding to >150% air satn.). Phase-B cells were harvested 24

h after 20 .mu.M 3-(3,4-dichlorophenyl)-1,1-dimethylurea had been added to the culture and gassing switched to 1% oxygen (<10 .mu.M). Phase-C cells originated from phase-B cells after 12 h of gassing the illuminated, yet non-growing cultures with air (21% oxygen or 200-220 .mu.M in the medium). Cytoplasmic membranes were isolated and purified from each of the three cell types. Non-covalently bound hemes were extd. and identified by reversed-phase high performance liq. chromatog. Besides ubiquitous heme B and heme A was detected in phase-A membranes while phase-B and phase-C membranes contained both hemes A and O, proportions of which depended on the oxygen status of the cells. CO/difference spectra, photo-action spectra of CO-inhibited oxygen uptake, and polarog. detn. of oxygen-affinities clearly showed that both hemes A and O were part of a functional form of cytochrome-c oxidase which, however, exhibited a single subunit-I **apoprotein** as verified by immunoblotting. Also electron transport characteristics did not give evidence for a quinol or any other alternate oxidase functioning in cyanobacteria.

L2 ANSWER 9 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:827922 CAPLUS
DOCUMENT NUMBER: 123:222476
TITLE: Occurrence of heme O in photoheterotrophically growing, semi-anaerobic cyanobacterium **Synechocystis** sp. PCC6803
AUTHOR(S): Peschek, Fuenter A.; Wastyn, Marnik; Fromwald, Susanne; Mayer, Bernhard
CORPORATE SOURCE: Biophysical Chemistry Group, University of Vienna, Vienna, Austria
SOURCE: FEBS Lett. (1995), 371(2), 89-93
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Extn. and identification of the non-covalently bound heme groups from crude membrane prepns. of photoheterotrophically grown **Synechocystis** sp. PCC 6803 by reversed-phase high performance liq. chromatog. and optical spectrophotometry led to the detection of heme O in addn. to hemes B and A, the latter of which was to be expected from the known presence of aa3-type cytochrome oxidase in cyanobacteria. In fully aerated cells (245 .mu.M dissolved O2 in the medium) besides heme B only heme A was found while in low-oxygen cells (<10 .mu.M dissolved O2) heme O was present at a concn. even higher than that of heme A. Given the possible role of heme O as a biosynthetic intermediate between heme B and heme A, together with generally much higher Km values of 5-50 .mu.M O2 for oxygenase as compared to Km values of 40-70 nM O2 for typical cytochrome-c oxidase, our findings allow the suggestion that the conversion of heme O to heme A is an obligately oxygen-requiring process catalyzed by some oxygenase directly introducing oxygen from

O2 into the 8-Me group of heme O. The occurrence of heme O (cytochrome o) in cyanobacteria does not imply the existence of an alternative oxidase since, according to the well-known promiscuity of heme groups, both hemes O and A are likely to combine with one and the same **apoprotein**.

L2 ANSWER 10 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:468152 CAPLUS
DOCUMENT NUMBER: 123:138445
TITLE: The role of cytochrome c-550 as studied through reverse genetics and mutant characterization in **Synechocystis** sp. PCC 6803
AUTHOR(S): Shen, Jian-Ren; Vermaas, Wim; Inoue, Yorinao
CORPORATE SOURCE: Institute Physical Chemical Research, RIKEN, Saitama, 351-01, Japan
SOURCE: J. Biol. Chem. (1995), 270(12), 6901-7
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The gene coding for cytochrome c-550 in **Synechocystis** sp. PCC 6803 was cloned based on the N-terminal sequence of the mature polypeptide. Using the most probable translation start codon, the gene is expected to code for 160 amino acid residues. This includes a cleavable N-terminal leader sequence of 25 residues. This leader sequence has an Arg-Asn-Arg sequence immediately before the cleavage site; this is characteristic for transit peptides in prokaryotes. Comparison of this sequence with the leader sequence of the photosystem II-assocd. extrinsic 33-kDa protein from the same cyanobacterium showed an identity of 13 out of 25 residues. These results suggest that after synthesis of the **apoprotein**, cytochrome c-550 is transported into the thylakoid lumen. Using the cloned gene, insertion and deletion mutants of **Synechocystis** sp. PCC 6803 were constructed. In the absence of cytochrome c-550, both mutants were capable of photoautotrophic growth but at a significantly reduced rate. Atrazine binding and Western blot anal. showed that these mutants on a per-chlorophyll basis contained 53-67% of the amt. of photosystem II as compared with wild type. The photosystem II-specific oxygen-evolving activity at satg. light intensity was reduced to about 40% of that in the wild type strain. Taken together, these results indicate that the cytochrome c-550 is transported into the thylakoid lumen and contributes to optimal functional stability of photosystem II in cyanobacteria. This supports our biochem. evidence that cytochrome c-550 is assocd. with the lumenal side of photosystem II as one of the extrinsic proteins enhancing oxygen evolution (Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1992) FEBS Lett. 301, 145-149; Shen, J.-R., and Inoue, Y. (1993) Biochem. 32, 1825-1832). Based on these results, the gene for cytochrome c-550 was named psbV. The possible evolutionary relationship among extrinsic proteins of the photosystem II donor

side is discussed.

L2 ANSWER 11 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1992:546832 CAPLUS
DOCUMENT NUMBER: 117:146832
TITLE: Phytochrome assembly. The structure and biological activity of 2(R),3(E)-phytochromobilin derived from phycobiliproteins
AUTHOR(S): Cornejo, Juan; Beale, Samuel I.; Terry, Matthew J.; Lagarias, J. Clark
CORPORATE SOURCE: Div. Biol. Med., Brown Univ., Providence, RI, 02912, USA
SOURCE: J. Biol. Chem. (1992), 267(21), 14790-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The unicellular rhodophyte Porphyridium cruentum and the filamentous cyanobacterium Calothrix sp. PCC 7601 contain phycobiliproteins that have covalently bound phycobilin chromophores. Overnight incubation of solvent-extd. cells at 40.degree. with methanol liberates free phycobilins that are derived from the protein-bound bilins by methanolytic cleavage of the thioether linkages between bilin and **apoprotein**. Two of the free bilins were identified as 3(E)-phycocyanobilin and 3(E)-phycoerythrobilin by comparative spectrophotometry and HPLC. Methanolysis also yields a third bilin free acid whose absorption and 1H NMR spectra support the assignment of the 3(E)-phytochromobilin structure. This novel bilin is the major pigment isolated from cells that are pre-extd. with acetone-contg. solvents. Since phytochrome- or phytochromobilin-contg. proteins are not present in either organism, the 3(E)-phytochromobilin must arise by oxidn. of phycobilin chromophores. This pigment is not obtained by similar treatment of a cyanobacterium and a rhodophyte that lack phycoerythrin. Therefore, 3(E)-phytochromobilin appears to be derived from phycoerythrobilin-contg. proteins. Comparative CD spectroscopy of 3(E)-phytochromobilin and 3(E)-phycocyanobilin suggests that the two bilins share the R stereochem. at the 2-position in the reduced pyrrole ring. Incubation of 2(R),3(E)-phytochromobilin with recombinant oat apophytochrome yields a covalent bilin adduct that is photoactive and spectrally indistinguishable from native oat phytochrome isolated from etiolated seedlings. Thus, phycobiliprotein-derived 2(R),3(E)-phytochromobilin is a biol. active phytochrome chromophore precursor.

L2 ANSWER 12 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1992:80607 CAPLUS
DOCUMENT NUMBER: 116:80607
TITLE: Regulation of photosynthetic membrane components in cyanobacteria

AUTHOR(S): Sherman, L. A.
CORPORATE SOURCE: Dep. Biol. Sci., Purdue Univ., Lafayette, IN, USA
SOURCE: Report (1991), DOE/ER/14028-2; Order No. DE91012713, 11 pp. Avail.: NTIS
From: Energy Res. Abstr. 1991, 16(8), Abstr. No. 21660
DOCUMENT TYPE: Report
LANGUAGE: English

AB Gene regulation was analyzed under different environmental conditions and the role of the psbO protein (MSP, the manganese stabilizing protein, the 33 kda protein) in O2-evolution was detd. These objectives are studied in the transformable cyanobacteria Synechococcus sp. PCC7942 and **Synechocystis** sp. PCC6803, resp. A deletion strain (.DELTA.psbO) was produced in **Synechocystis** that completely lacks the gene or the gene product, and which can grow photosynthetically at about 2/3 the normal rate. This deletion strain was used to construct site-directed mutations at specific, externally-located, charged residues. In particular, residues and domains that enable MSP to interact with the photosystem II (PSII) reaction center components were analyzed. Genes were identified and cloned which code for the novel chlorophyll-protein complex which is synthesized during iron-deficiency. The **apoprotein**, isiA, has an amino acid sequence very similar to that of the gene product of psbC (CP43) except for the lack of most of the large lumenal loop E. An insertion mutant in isiA was found, which can grow normally in regular iron-sufficient medium and in partially iron-deficient cultures. A putative candidate or irpR, the iron-regulated DNA-binding protein that is used to control gene expression based on iron concns. was identified. Gene regulation in iron-deficient conditions, as well as the role of isiA in PSII function and in membrane assembly are discussed.

L2 ANSWER 13 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1992:55740 CAPLUS
DOCUMENT NUMBER: 116:55740
TITLE: Targeted genetic inactivation of the photosystem I reaction center in the cyanobacterium **Synechocystis** sp. PCC 6803
AUTHOR(S): Smart, Lawrence B.; Anderson, Shawn L.; McIntosh, Lee
CORPORATE SOURCE: DOE-Plant Res. Lab., Michigan State Univ., East Lansing, MI, 48824, USA
SOURCE: EMBO J. (1991), 10(11), 3289-96
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This report describes the first complete segregation of a targeted

inactivation of psaA encoding one of the P700-chlorophyll a **apoproteins** of photosystem (PS) I. A kanamycin resistance gene was used to interrupt the psaA gene in the unicellular cyanobacterium **Synechocystis** sp. PPC 6803. Selection of a fully segregated mutant, ADK9, was performed under light-activated heterotrophic growth (LAHG) conditions; complete darkness except for 5 min of light every 24 h and 5 mM glucose. Under these conditions, wild-type cells showed a 4-fold decrease in chlorophyll (chl) per cell, primarily due to a decrease of PS I reaction centers. Evidence for the absence of PS I in ADK9 includes: the lack of EPR signal I, from P700+; undetectable P700-**apoprotein**; greatly reduced whole-chain photosynthesis rates; and greatly reduced chl per cell, resulting in a turquoise blue phenotype. The PS I peripheral proteins PSA-C and PSA-D were not detected in this mutant. ADK9 does assemble near wild-type levels of functional PS II per cell, evidenced by: EPR signal II from YD+; high rates of oxygen evolution with 2,6-dichloro-p-benzoquinone (DCBQ), an electron acceptor from PS II; and accumulation of D1, a PS II core polypeptide. The success of this transformation indicates that this cyanobacterium may be utilized for site-directed mutagenesis of the PS I core.

L2 ANSWER 14 OF 14 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1989:474964 CAPLUS
DOCUMENT NUMBER: 111:74964
TITLE: Visualization of antibody binding to the photosynthetic membrane: the transmembrane orientation of cytochrome b-559
AUTHOR(S): Vallon, Olivier; Tae, Gun Sik; Cramer, William A.; Simpson, David; Hoyer-Hansen, Gunilla; Bogorad, Lawrence
CORPORATE SOURCE: Dep. Cell. Dev. Biol., Harvard Univ., Cambridge, MA, USA
SOURCE: Biochim. Biophys. Acta (1989), 975(1), 132-41
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Immuno-gold labeling and electron microscopy were used to study topog. of thylakoid membrane polypeptides. Thylakoid vesicles formed by passage through a French press were adsorbed onto a plastic film supported by an electron microscope grid and processed for single or double immuno-gold labeling. After shadowing with platinum, the inside-out and right-side-out vesicles were identified by their distinctive morphologies. Right-side-out vesicles were labeled by a monoclonal antibody recognizing an epitope located in the trypsin-cleaved, N-terminal portion of the photosystem II light-harvesting complex **apopr tein**, and by an antibody to CF1. A monoclonal antibody to the .alpha.-subunit of cytochrome b-559 reacted with a synthetic tridecapeptide corresponding to the

C-terminal portion of the polypeptide. Both this antibody and a polyclonal antibody to the synthetic peptide labeled inside-out vesicles exclusively, indicating that the polypeptide C-terminus was exposed on the lumenal (exoplasmic) surface of the membrane.

=> d his l4-; d 1-33 ibib abs

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, BIOTECHDS, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT' ENTERED AT 14:13:36 ON 13 SEP 1999)
L4 62 S L1 AND (SYNECHOCYST? OR CPH2 OR CPH 2)
L5 33 DUP REM L4 (29 DUPLICATES REMOVED)

L5 ANSWER 1 OF 33 MEDLINE
ACCESSION NUMBER: 1999162261 MEDLINE
DOCUMENT NUMBER: 99162261
TITLE: Mutation of Phe-363 in the photosystem II protein CP47 impairs photoautotrophic growth, alters the chloride requirement, and prevents photosynthesis in the absence of either PSII-O or PSII-V in **Synechocystis** sp. PCC 6803.
AUTHOR: Clarke S M; Eaton-Rye J J
CORPORATE SOURCE: Department of Biochemistry, University of Otago, Dunedin, New Zealand.
SOURCE: BIOCHEMISTRY, (1999 Mar 2) 38 (9) 2707-15.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199905
ENTRY WEEK: 19990504
AB The deletion of the amino acids between Gly-351 and Thr-365 within the large, lumen-exposed, hydrophilic region (loop E) of the photosystem II (PSII) chlorophyll a-binding protein CP47 produced a strain of **Synechocystis** sp. PCC 6803 that failed to assemble stable PSII centers [Eaton-Rye, J. J., and Vermaas, W. F. J. (1991) Plant Mol. Biol. 17, 1165-1177]. The importance of two conserved Phe residues at positions 362 and 363 within this deletion has been investigated. The F363R strain had impaired photoautotrophic growth and an enhanced sensitivity to photoinactivation, demonstrating that Phe is required at position 363 for normal PSII function. In contrast, photoautotrophic growth in strains N361K and F362R was unaffected. Uniquely, among the mutant strains tested, F363R was unable to grow under chloride-limiting conditions, and this effect was reversed by replacing chloride with bromide. The removal of the manganese-stabilizing protein (PSII-O), the 12 kDa extrinsic protein (PSII-U), and cytochrome c-550 (PSII-V) was investigated in each mutant in vivo. In N361K and F362R, removal of PSII-V produced a

more deleterious effect than the removal of PSII-O, but even so, all strains remained photoautotrophic. In contrast, the absence of PSII-V and PSII-O in F363R produced obligate photoheterotrophic strains. The removal of PSII-U increased the susceptibility of PSII to heat inactivation and further decreased the stability of PSII in F363R, demonstrating that PSII-U can contribute to the stabilization of mutations that have been introduced into CP47. The order of importance of the selective removal of the extrinsic proteins in strains carrying mutations in loop E of CP47 was found to be as follows: DeltaPSII-V >/= DeltaPSII-O > DeltaPSII-U.

L5 ANSWER 2 OF 33 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999306841 MEDLINE
DOCUMENT NUMBER: 99306841
TITLE: Extended heme promiscuity in the cyanobacterial cytochrome c oxidase: characterization of native complexes containing hemes A, O, and D, respectively.
AUTHOR: Fromwald S; Zoder R; Wastyn M; Lubben M; Peschek G A
CORPORATE SOURCE: Molecular Bioenergetics Group, Institute of Physical Chemistry, University of Vienna, Wien, A-1090, Austria.
SOURCE: ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, (1999 Jul 1) 367 (1) 122-8.
Journal code: 6SK. ISSN: 0003-9861.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199909
ENTRY WEEK: 19990903
AB The cyanobacteria Anacystis nidulans (Synechococcus sp. PCC6301), **Synechocystis** sp. PCC6803, Anabaena sp. PCC 7120, and Nostoc sp. PCC8009 were grown photoautotrophically under reduced oxygen tension in a medium with sulfate replaced by thiosulfate and nitrate replaced by ammonium as the S- and N-sources, respectively. In addition, Anabaena and Nostoc were grown under dinitrogen-fixing conditions in a medium free of combined nitrogen. Membranes were isolated from late-logarithmic cells (culture density corresponding to approximately 3 microliters packed cells per milliliter); cytoplasmic and thylakoid membranes were separated and purified according to established procedures. Acid-labile hemes were extracted from the membranes and subjected to reversed-phase high-performance liquid chromatography. Separated hemes were analyzed spectroscopically and identified by comparison with authentic standards. In addition to hemes B, A, and O, the latter of which was induced under semianaerobic conditions only, substitution of thiosulfate and ammonium for the oxy-anions sulfate and nitrate led to the appearance of spectrally discernible heme D in the membranes and extracts therefrom. However, spectroscopic and kinetic

investigation of the membrane-bound heme D rather disproved any reaction with oxygen or carbon monoxide. Kinetic measurements performed with the membrane-bound respiratory oxidase gave evidence for only two kinetically competent terminal oxidases, a3 and o3, both apparently associated with a single type of **apoprotein**, viz. subunit I of the known cyanobacterial aa3-type cytochrome c oxidase. The heme D, on the other hand, seems to form a spectrally distinguished, yet kinetically ill-defined hemoprotein complex which does not qualify as a fully functional d-type terminal oxidase on our (wild-type) cyanobacteria even after growth under semianaerobic pseudo-reducing conditions. Also growth (of Anabaena and Nostoc) under dinitrogen-fixing conditions did not change this situation. Thus, we are left with (wild-type) cyanobacteria forming an unbranched respiratory chain with only a single type of terminal oxidase protein, viz. the known aa3-type cytochrome c oxidase. This oxidase, however, may incorporate different prosthetic (heme) groups in the sense of "heme promiscuity." Biosynthesis of the different heme groups thereby seems to respond to the ambient redox environment. In particular, however, conditions for expression of the two quinol oxidases potentially and additionally coded for by the genome of, e. g., **Synechocystis** sp. PCC6803 (see http://www.kazusa.or.jp/cyano), have not yet been found. Copyright 1999 Academic Press.

L5 ANSWER 3 OF 33 MEDLINE
ACCESSION NUMBER: 1998447515 MEDLINE
DOCUMENT NUMBER: 98447515
TITLE: Specific requirements for cytochrome c-550 and the manganese-stabilizing protein in photoautotrophic strains of **Synechocystis** sp. PCC 6803 with mutations in the domain Gly-351 to Thr-436 of the chlorophyll-binding protein CP47.
AUTHOR: Morgan T R; Shand J A; Clarke S M; Eaton-Rye J J
CORPORATE SOURCE: Department of Biochemistry, University of Otago, Dunedin, New Zealand.
SOURCE: BIOCHEMISTRY, (1998 Oct 13) 37 (41) 14437-49. Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104
AB The requirement of cytochrome c-550 (PSII-V) in photosystem II (PSII) has been assessed in **Synechocystis** sp. PCC 6803 containing mutations between Gly-351 and Thr-436 of the loop E domain of the chlorophyll a-binding protein CP47. Six photoautotrophic strains were utilized to compare the effect of removal of either the manganese-stabilizing protein (PSII-O) or

PSII-V on PSII activity in vivo. These were a wild-type control; two strains with amino acid deletions, Delta(R384-V392) and Delta(G429-T436); and three carrying specific amino acid substitutions, G351L/T365Q, G351L/E364Q/T365Q, and G351L/E353Q/E355Q/T365Q. The removal of PSII-O prevented the assembly of PSII in Delta(G429-T436) but not in Delta(R384-V392). Neither Delta(G429-T436) nor Delta(R384-V392) could support photoautotrophic growth in the absence of PSII-V. In chloride-limiting conditions, the photoautotrophic growth of Delta(R384-V392) was severely impaired and that of Delta(G429-T436) totally inhibited, and no strains lacking PSII-V could grow in chloride-limiting or calcium-limiting media. Substitutions at Gly-351, Glu-353, Glu-355, and Thr-365 produced phenotypes that were similar to those of the control in the presence or absence of PSII-O and PSII-V, but removal of PSII-O from G351L/E364Q/T365Q produced a significant reduction of assembled PSII centers and an enhanced sensitivity to photoinactivation while removal of PSII-V prevented photoautotrophic growth. The additional mutants E364Q:DeltaPSII-V and E364G:DeltaPSII-V demonstrated that this inhibition was a consequence of the mutation at Glu-364. These results also show that the removal of PSII-V, in vivo, produces phenotypes in the CP47 mutants examined that are either similar or more severe than those resulting from the removal of PSII-O.

L5 ANSWER 4 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 1998:377127 SCISEARCH
THE GENUINE ARTICLE: ZM977
TITLE: Chlorophyll a availability affects psbA translation and D1 precursor processing in vivo in **Synechocystis** sp. PCC 6803
AUTHOR: He Q F; Vermaas W (Reprint)
CORPORATE SOURCE: ARIZONA STATE UNIV, DEPT PLANT BIOL, BOX 871601, TEMPE, AZ 85287 (Reprint); ARIZONA STATE UNIV, DEPT PLANT BIOL, TEMPE, AZ 85287; ARIZONA STATE UNIV, CTR STUDY EARLY EVENTS PHOTOSYNTHESIS, TEMPE, AZ 85287
COUNTRY OF AUTHOR: USA
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (12 MAY 1998) Vol. 95, No. 10, pp. 5830-5835.
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418.
ISSN: 0027-8424.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 47
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Transcript accumulation and translation of psbA as well as processing of the D1 precursor protein were investigated in relation

to chlorophyll availability in vivo in cyanobacterial strains lacking photosystem I (PS I). The psbA transcript level was almost independent of chlorophyll availability and was approximate to 3-fold Bower in darkness than in continuous light (5 mu E m(-2) s(-1)). Upon illumination, it reached a steady-state level within several hours, Upon growth under light-activated heterotrophic growth conditions (LAHG) in the EPS I-less strain, D1 synthesis occurred immediately upon illumination, However, in FS I-less/chlL(-) cells, which lacked the light-independent chlorophyll biosynthesis pathway and had very low chlorophyll levels after LAHG growth, very little D1 synthesis occurred upon illumination, and the synthesis rate increased with time. This result suggests a translational control of D1 biosynthesis related to chlorophyll availability, Upon illumination, initially a high Bevel of the nonprocessed D1 precursor was observed by pulse labeling and immunodetection in LAHG-grown PS I-less/chlL(-) cells but not in PS I-less cells. A significant amount of the D1 precursor eventually was processed to mature DB, and the half-life of the D1 precursor decreased as the chlorophyll content of the cells increased. The Ell processing enzyme CtpA was found to be present at similar levels regardless illumination or chlorophyll levels. We conclude that, directly or indirectly. chlorophyll availability is needed for HPI translation as well as for efficient professing of the D1 precursor.

L5 ANSWER 5 OF 33 MEDLINE
ACCESSION NUMBER: 1998145216 MEDLINE
DOCUMENT NUMBER: 98145216
TITLE: Functional analysis of combinatorial mutants altered in a conserved region in loop E of the CP47 protein in **Synechocystis** sp. PCC 6803.
AUTHOR: Tichy M; Vermaas W
CORPORATE SOURCE: Department of Plant Biology, Arizona State University, Tempe 85287-1601, USA.
SOURCE: BIOCHEMISTRY, (1998 Feb 10) 37 (6) 1523-31. Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY WEEK: 19980502
AB Regions in the large lumenally exposed region (loop E) of CP47 affect properties of the watersplitting system in photosystem II (PS II). To investigate the role of these regions, we developed a method for functional complementation of obligate photoheterotrophic mutants carrying a deletion in one such region. Using an obligate photoheterotrophic mutant that carries a short deletion (delta (D440-P447) in loop E of CP47, completely degenerate sequences of eight codons in length were introduced at the site of the deletion.

Transformants that were complemented to photoautotrophic growth were selected, and 20 such mutants were studied. Sequence analysis revealed that, as expected, in each of them CP47 had been restored to its wild-type length. However, none of the amino acid residues in the deleted region were found to be critical for function. A negatively charged residue at position 440 and a positively charged one at position 444 were favored but not required. Photoautotrophic growth of mutants obtained varied from almost normal to significantly impaired. The mutants contained 20-100% of the amount of PS II present in the wild type, with PS II amounts correlating with the initial rates of oxygen evolution. The mutants had a high rate of photoinactivation, and many mutants showed an up to 1000-fold increase in chloride requirement for photoautotrophic growth. These phenotypic effects were a direct consequence of the CP47 mutations and were not caused by altered binding of one of the extrinsic proteins. No particular amino acid residues in positions 440-447 of CP47 were found to be indispensable for photoautotrophic growth, and many amino acid combinations in this region support PS II function. However, the mutagenized region is shown to interact with the oxygen-evolving site of PS II and appears to have a direct role in chloride binding.

L5 ANSWER 6 OF 33 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999306316 MEDLINE
DOCUMENT NUMBER: 99306316
TITLE: The system of phytochromes: photobiophysics and photobiochemistry in vivo.
AUTHOR: Sineshchekov V A
CORPORATE SOURCE: Department of Biology, Lomonosov Moscow State University, Russia.. vitally@vsineshchekov.home.bio.msu.ru
SOURCE: MEMBRANE AND CELL BIOLOGY, (1998) 12 (5) 691-720.
Ref: 110
Journal code: CWK. ISSN: 1023-6597.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY WEEK: 19990905
AB Phytochrome is a key photoregulation pigment in plants which determines the strategy of their development throughout their life cycle. The major achievement in the recent investigations of the pigment is the discovery of its structural and functional heterogeneity: existence of a family of phytochromes (phyA-phyE) differing by the **apoprotein** was demonstrated. We approach this problem by investigating the chromophore component of the

pigment with the use of the developed method of in vivo low-temperature fluorescence spectroscopy of phytochrome. In etiolated plants, phytochrome fluorescence was detected and attributed to its red-light absorbing form (Pr) and the first photoproduct (lumi-R), and a scheme of the photoreaction in phytochrome, a distinction of which is the activation barrier in the excited state, was put forward. It was found that the spectroscopic and photochemical characteristics of Pr depend on the plant species and phytochrome mutants and overexpressors used, on localization of the pigment in organs and tissues, plant age, effect of preillumination and other physiological factors. This variability of the parameters was interpreted as the existence of at least two phenomenological Pr populations, which differ by their spectroscopic characteristics and activation parameters of the Pr --> lumi-R photoreaction (in particular, by the extent of the Pr --> lumi-R photoconversion at low temperatures, gamma1): the longer-wavelength major and variable by its content in plant tissues Pr' with gamma1 = 0.5 and the shorter-wavelength minor relatively constant Pr" with gamma1 < or = 0.05. The analysis of the phytochrome mutants and overexpressors allows a conclusion that phytochrome A (phyA), which dominates in etiolated seedlings, is presented by two isoforms attributed to Pr' and Pr" (phyA' and phyA", respectively). Phytochrome B (phyB) accounts for less than 10% of the total phytochrome fluorescence and belongs to the Pr" type. It is also characterized by the relatively low extent of the Pr photoconversion into the far-red-light absorbing physiologically active phytochrome form, Pfr. Fluorescence of the minor phytochromes (phyC-phyE) is negligible. The recently discovered phytochrome of the cyanobacterium **Synechocystis** also belongs to the phenomenological Pr" type. PhyA' is a light-labile and soluble fraction, while phyA" is a relatively light-stable and, possibly, membrane (protein)-associated. Experiments with transgenic tobacco plants overexpressing full-length and C- and N-terminally truncated oat phytochrome A suggest that phyA' and phyA" might differ by the post-translational modification of the small N-terminal segment (amino acid residues 7-69) of the pigment. PhyA' is likely to be active in the de-etiolation processes while phyA" together with phyB, in green plants as revealed by the experiments on transgenic potato plants and phytochrome mutants of Arabidopsis and pea with altered levels of phytochromes A and B and modified phenotypes. And finally, within phyA', there are three subpopulations which are, possibly, different conformers of the chromophore. Thus, there is a hierarchical system of phytochromes which include: (i) different phytochromes; (ii) their post-translationally modified states and (iii) conformers within one molecular type. Its existence might be the rationale for the multiplicity of the photoregulation reactions in plants mediated by phytochrome.

L5 ANSWER 7 OF 33 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 3
Searcher : Shears 308-4994

ACCESSION NUMBER: 1999:35584 BIOSIS
DOCUMENT NUMBER: PREV199900035584
TITLE: The system of phytochromes: Photobiophysics and photobiochemistry in vivo.
AUTHOR(S): Sineshchekov, V. A. (1)
CORPORATE SOURCE: (1) Fac. Biol., M. V. Lomonosov Mosc. State Univ., Moscow 119899 Russia
SOURCE: Biologicheskie Membrany (Moscow), (Sept.-Oct., 1998) Vol. 15, No. 5, pp. 549-572.
ISSN: 0233-4755.
DOCUMENT TYPE: Article
LANGUAGE: Russian
SUMMARY LANGUAGE: Russian; English
AB The major achievement in the recent investigations of phytochrome, a key photoregulation pigment in plants, is the discovery of its structural and functional heterogeneity. A small family of phytochromes which differ by the **apoprotein** was detected by means of immunochemistry, molecular biology and genetics. We have been approaching this problem by investigating the chromophore component of the pigment by means of the developed method of in-vivo low-temperature fluorescence spectroscopy of phytochrome. In etiolated plants, phytochrome fluorescence was detected and attributed to its red-light absorbing form (Pr) and the first photoproduct (lumi-R). A scheme of the photoreaction in phytochrome, a distinction of which is the activation barrier in the excited state, was put forward. The spectroscopic and photochemical characteristics of Pr were found to depend on the plant species and phytochrome mutants and overexpressors used, on localization of the pigment in organs and tissues, plant age, effect of preillumination and other physiological factors. This variability of the parameters was interpreted as the existence of at least two phenomenological Pr populations, which differ by their spectroscopic characteristics and activation parameters of the Pr fwdarw lumi-R photoreaction (in particular, by the extent of the Pr fwdarw lumi-R photoconversion at low (85 K) temperatures, gamma1): the longer wavelength major Pr', which is variable by its content in plant tissues, with gamma1 = 0.5 and the shorter wavelength minor relatively constant Pr" with gamma1 ltoreq 0.05. The analysis of the phytochrome mutants and overexpressors allows a conclusion that phytochrome A (phyA), which dominates in etiolated seedlings, is represented by two isoforms attributed to Pr' and Pr" (phyA' and phyA", respectively). Phytochrome B (phyB) accounts for less than 10% of the total phytochrome fluorescence. It belongs to the Pr" type and is also characterized by a relatively low photoconversion into the far-red-light absorbing physiologically active phytochrome form, Pfr. Fluorescence of the minor phytochromes (phyC-phyE) is negligible. The recently discovered phytochrome of the cyanobacterium **Synechocystis** also belongs to the phenomenological Pr" type. PhyA' is a light-labile and soluble

fraction, while phyA" is a relatively light-stable and, possibly, membrane (protein)-associated. Experiments with transgenic tobacco plants overexpressing full-length and C- and N-terminally truncated oat phytochrome A suggest that phyA' and phyA' differ by the post-translational modification of the small N-terminal segment (amino acid residues 7-69) of the pigment. PhyA' is likely to be active in the de-etiolation processes while phyA", together with phyB, in green plants as revealed by the experiments on transgenic potato plants and phytochrome mutants of Arabidopsis and pea with altered levels of phytochromes A and B and modified phenotypes. And finally, within phyA, there are three subpopulations which are, possibly, different conformers of the chromophore. Thus, there is a hierarchical system of phytochromes which include: 1) different phytochromes; 2) their post-translationally modified states and 3) conformers within one molecular type. Its existence might be the rationale for the multiplicity of the photoregulation reactions in plants mediated by phytochrome.

L5 ANSWER 8 OF 33 MEDLINE
ACCESSION NUMBER: 1998422604 MEDLINE
DOCUMENT NUMBER: 98422604
TITLE: Department of Biological Sciences, Louisiana State University, Baton Rouge 70803, USA.
AUTHOR: Bricker T M; Putnam-Evans C; Wu J
CORPORATE SOURCE: Directed mutagenesis in photosystem II: analysis of the CP 47 protein.
SOURCE: METHODS IN ENZYMOLOGY, (1998) 297 320-37.
Journal code: MVA. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY WEEK: 19990104

L5 ANSWER 9 OF 33 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998148783 MEDLINE
DOCUMENT NUMBER: 98148783
TITLE: Fluorescence and photochemistry of recombinant phytochrome from the cyanobacterium **Synechocystis**.
AUTHOR: Sineshchekov V; Hughes J; Hartmann E; Lamparter T
CORPORATE SOURCE: Biology Department, M. V. Lomonosov Moscow State University, Russia.. vitally@VSineshchekov.home.bio.msu.ru
SOURCE: PHOTOCHEMISTRY AND PHOTOBIOLOGY, (1998 Feb) 67 (2) 263-7.
Journal code: P69. ISSN: 0031-8655.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
ENTRY MONTH: 199806
ENTRY WEEK: 19980601

AB Fluorescence and photochemical properties of phytochrome from the cyanobacterium **Synechocystis** were investigated in the temperature interval from 293 to 85 K. The **apoprotein** was obtained by overexpression in Escherichia coli and assembled to a holophytochrome with phycocyanobilin (PCB) and phytochromobilin (P phi B), Syn(PCB)phy and Syn(P phi B)phy, respectively. Its red-absorbing form, Pr, is characterized at 85 K by the emission and excitation maxima at 682 and 666 nm in Syn(PCB)phy and at 690 and 674 nm in Syn(P phi B)phy. At room temperature, the spectra are blue shifted by 5-10 nm. The fluorescence intensity dropped down by approximately 15-20-fold upon warming from 85 to 293 K and activation energy of the fluorescence decay was estimated to be ca 5.4 and 4.9 kJ mol-1 in Syn(PCB)phy and Syn(P phi B)phy, respectively. Phototransformation of Pr upon red illumination was observed at temperatures above 160-170 K in Syn(PCB)phy and above 140-150 K in Syn(P phi B)phy with a 2-3 nm shift of the emission spectrum to the blue and increase of the intensity of its shorter wavelength part. This was interpreted as a possible formation of the photoproduct of the meta-Ra type of the plant phytochrome. At ambient temperatures, the extent of the Pr phototransformation to the far-red-absorbing form, Pfr, was ca 0.7-0.75 and 0.85-0.9 for Syn(PCB)phy and Syn(P phi B)phy, respectively. Fluorescence of Pfr and of the photoproduct similar to lumi-R was not observed. With respect to the photochemical parameters, Syn(PCB)phy and Syn(P phi B)phy are similar to each other and also to a small fraction of phyA (phyA") and to phyB. The latter were shown to have low photochemical activity at low temperatures in contrast to the major phyA pool (phyA'), which is distinguished by the high extent (ca 50%) of Pr phototransformation at 85 K. These photochemical features are interpreted in terms of different activation barriers for the photoreaction in the Pr excited state.

L5 ANSWER 10 OF 33 MEDLINE
ACCESSION NUMBER: 1999023730 MEDLINE
DOCUMENT NUMBER: 99023730
TITLE: Isolation of a highly active photosystem II preparation from **Synechocystis** 6803 using a histidine-tagged mutant of CP 47.
AUTHOR: Bricker T M; Morvant J; Masri N; Sutton H M; Frankel L K
CORPORATE SOURCE: Department of Biological Sciences, Biochemistry and Molecular Biology Section, Louisiana State University, Baton Rouge, LA 70803, USA.. btbric@lsuvm.sncc.lsu.edu
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1998 Nov 2) 1409 (1)
Searcher : Shears 308-4994

50-7.
Journal code: A0W. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199902
ENTRY WEEK: 19990204

AB Site-directed mutagenesis was used to produce a **Synechocystis** mutant containing a histidine tag at the C terminus of the CP 47 protein of Photosystem II. This mutant cell line, designated HT-3, exhibited slightly above normal rates of oxygen evolution and appeared to accumulate somewhat more Photosystem II reaction centers than a control strain. A rapidly isolatable (<7 h) oxygen-evolving Photosystem II preparation was prepared from HT-3 using dodecyl-beta-d-maltoside solubilization and Co2+ metal affinity chromatography. This histidine-tagged Photosystem II preparation stably evolved oxygen at a high rate (2440 micromol O2 (mg chl)-1 h-1), exhibited an alpha-band absorption maximum at 674 nm, and was highly enriched in a number of Photosystem II components including cytochrome c550. Fluorescence yield analysis using water or hydroxylamine as an electron donor to the Photosystem II preparation indicated that virtually all of the Photosystem II reaction centers were capable of evolving oxygen. Proteins associated with Photosystem II were highly enriched in this preparation. 3,3',5, 5'-Tetramethylbenzidine staining indicated that the histidine-tagged preparation was enriched in cytochromes c550 and b559 and depleted of cytochrome f. This result was confirmed by optical difference spectroscopy. This histidine-tagged Photosystem II preparation may be very useful for the isolation of Photosystem II preparations from mutants containing lesions in other Photosystem II proteins.

L5 ANSWER 11 OF 33 MEDLINE
ACCESSION NUMBER: 1998002715 MEDLINE
DOCUMENT NUMBER: 98002715
TITLE: Raman spectroscopic and light-induced kinetic characterization of a recombinant phytochrome of the cyanobacterium **Synechocystis**.
AUTHOR: Remberg A; Lindner I; Lamparter T; Hughes J; Kneip C; Hildebrandt P; Braslavsky S E; Gartner W; Schaffner K
CORPORATE SOURCE: Max-Planck-Institut fur Strahlenchemie, Postfach 101365, D-45413 Mulheim an der Ruhr, Germany.
SOURCE: BIOCHEMISTRY, (1997 Oct 28) 36 (43) 13389-95.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199801
ENTRY WEEK: 19980104
AB A phytochrome-encoding cDNA from the cyanobacterium **Synechocystis** has been heterologously expressed in Escherichia coli and reconstituted into functional chromoproteins by incubation with either phycocyanobilin (PCB) or phytochromobilin (PPhiB). These materials were studied by Raman spectroscopy and nanosecond flash photolysis. The Raman spectra suggest far-reaching similarities in chromophore configuration and conformation between the Pfr forms of **Synechocystis** phytochrome and the plant phytochromes (e.g. phyA from oat), but some differences, such as torsions around methine bridges and in hydrogen bonding interactions, in the Pr state. **Synechocystis** phytochrome (PCB) undergoes a multistep photoconversion reminiscent of the phyA Pr --> Pfr transformation but with different kinetics. The first process resolved is the decay of an intermediate with red-shifted absorption (relative to parent state) and a 25-micros lifetime. The next observable intermediate grows in with 300 (+/-25) micros and decays with 6-8 ms. The final state (Pfr) is formed biexponentially (450 ms, 1 s). When reconstituted with PPhiB, the first decay of this **Synechocystis** phytochrome is biexponential (5 and 25 micros). The growth of the second intermediate is slower (750 micros) than that in the PCB adduct whereas the decays of both species are similar. The formation of the Pfr form required fitting with three components (350 ms, 2.5 s, and 11 s). H/D Exchange in **Synechocystis** phytochrome (PCB) delays, by an isotope effect of 2.7, both growth (300 micros) and decay rates (6-8 ms) of the second intermediate. This effect is larger than values determined for phyA (ca. 1.2) and is characteristic of a rate-limiting proton transfer. The formation of the Pfr state of the PCB adduct of **Synechocystis** phytochrome shows a deuterium effect similar as phyA (ca. 1.2). Activation energies of the second intermediate in the range 0-18 degrees C are 44 (in H2O/buffer) and 48 kJ mol-1 (D2O), with essentially identical pre-exponential factors.

L5 ANSWER 12 OF 33 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998004477 MEDLINE
DOCUMENT NUMBER: 98004477
TITLE: Characterization of recombinant phytochrome from the cyanobacterium **Synechocystis** [published erratum appears in Proc Natl Acad Sci U S A 1998 Mar 3;95(5):2714].
AUTHOR: Lamparter T; Mittmann F; Gartner W; Borner T; Hartmann E; Hughes J
CORPORATE SOURCE: Institut fur Pflanzenphysiologie und Mikrobiologie, Freie Universitat, Konigin-Luise-Strasse 12-16, D-14195 Berlin, Germany.. lamparte@zedat.fu-berlin.de
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Oct 28) 94 (22)

11792-7.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
OTHER SOURCE: GENBANK-D28242; GENBANK-U56698; GENBANK-U59741; GENBANK-U67397
ENTRY MONTH: 199802

AB The complete sequence of the **Synechocystis** chromosome has revealed a phytochrome-like sequence that yielded an authentic phytochrome when overexpressed in Escherichia coli. In this paper we describe this recombinant **Synechocystis** phytochrome in more detail. Islands of strong similarity to plant phytochromes were found throughout the cyanobacterial sequence whereas C-terminal homologies identify it as a likely sensory histidine kinase, a family to which plant phytochromes are related. An approximately 300 residue portion that is important for plant phytochrome function is missing from the **Synechocystis** sequence, immediately in front of the putative kinase region. The recombinant **apoprotein** is soluble and can easily be purified to homogeneity by affinity chromatography. Phycocyanobilin and similar tetrapyrroles are covalently attached within seconds, an autocatalytic process followed by slow conformational changes culminating in red-absorbing phytochrome formation. Spectral absorbance characteristics are remarkably similar to those of plant phytochromes, although the conformation of the chromophore is likely to be more helical in the **Synechocystis** phytochrome. According to size-exclusion chromatography the native recombinant **apoproteins** and holoproteins elute predominantly as 115- and 170-kDa species, respectively. Both tend to form dimers in vitro and aggregate under low salt conditions. Nevertheless, the purity and solubility of the recombinant gene product make it a most attractive model for molecular studies of phytochrome, including x-ray crystallography.

L5 ANSWER 13 OF 33 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998048479 MEDLINE
DOCUMENT NUMBER: 98048479
TITLE: Characterization of a gene encoding dihydrolipoamide dehydrogenase of the cyanobacterium **Synechocystis** sp. strain PCC 6803.
AUTHOR: Engels A; Pistorius E K
CORPORATE SOURCE: Universitat Bielefeld, Germany.
SOURCE: MICROBIOLOGY, (1997 Nov) 143 (Pt 11) 3543-53.
Journal code: BXW. ISSN: 1350-0872.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z48564
ENTRY MONTH: 199804
ENTRY WEEK: 19980402

AB The authors previously reported the isolation and partial characterization of a periplasmically located dihydrolipoamide dehydrogenase (LPD) from the cyanobacterium **Synechocystis** sp. strain PCC 6803. In the present work the gene (lpdA; database accession number Z48564) encoding the **apoprotein** of this LPD in **Synechocystis** PCC 6803 has been identified, sequenced and analysed. The lpdA gene codes for a protein starting with methionine, which is post-translationally removed. The mature protein contains an N-terminal serine and consists of 473 amino acids with a deduced molecular mass of 51421 Da (including one FAD). The LPD is an acidic protein with a calculated isoelectric point of 5.17. Comparison of the amino acid sequence of the **Synechocystis** LPD with protein sequences in the databases revealed that the enzyme shares identities of 31-35% with all 18 LPDs so far sequenced and published. As a first step in determining the role of this cyanobacterial LPD, attempts were made to generate an LPD-free **Synechocystis** mutant by insertionally inactivating the lpdA gene with a kanamycin-resistance cassette. However, the selected transformants appeared to be heteroallelic, containing both the intact lpdA gene and the lpdA gene inactivated by the drug-resistance cassette. The heteroallelic mutant studied, which had about 50% of the wild-type LPD activity, caused acidification of the growth medium. Growth over a prolonged time was only possible after an increased buffering of the medium. Since it is reported in the literature that inactivation of the pyruvate dehydrogenase complex (PDC) leads to acidosis, a function of the LPD in a cytoplasmic-membrane-associated PDC is conceivable.

L5 ANSWER 14 OF 33 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:42191 BIOSIS
DOCUMENT NUMBER: PREV199800042191
TITLE: The phytofluors: A new class of fluorescent protein probes.
AUTHOR(S): Murphy, John T.; Lagarias, J. Clark (1)
CORPORATE SOURCE: (1) Sect. Mol. Cell. Biol., Univ. Calif., One Shields Ave., Davis, CA 95616 USA
SOURCE: Current Biology, (Nov., 1997) Vol. 7, No. 11, pp. 870-876.
ISSN: 0960-9822.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Background: Biologically compatible fluorescent protein probes, particularly the self-assembling green fluorescent protein (GFP) from the jellyfish Aequorea victoria, have revolutionized research in cell, molecular and developmental biology because they allow

visualization of biochemical events in living cells. Additional fluorescent proteins that could be reconstituted in vivo while extending the useful wavelength range towards the orange and red regions of the light spectrum would increase the range of applications currently available with fluorescent protein probes. Results: Intensely orange fluorescent adducts, which we designate phytofluors, are spontaneously formed upon incubation of recombinant plant phytochrome **apoproteins** with phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin chromophore. Phytofluors have large molar absorption coefficients, fluorescence quantum yields greater than 0.7, excellent photostability, stability over a wide range of pH, and can be reconstituted in living plant cells. Conclusions: The phytofluors constitute a new class of fluorophore that can potentially be produced upon bilin uptake by any living cell expressing an apophytochrome cDNA. Mutagenesis of the phytochrome **apoprotein** and/or alteration of the linear tetrapyrrole precursor by chemical synthesis are expected to afford new phytofluors with fluorescence excitation and emission spectra spanning the visible to near-infrared light spectrum.

L5 ANSWER 15 OF 33 MEDLINE
ACCESSION NUMBER: 97369368 MEDLINE
DOCUMENT NUMBER: 97369368
TITLE: Site-directed mutagenesis of the basic residues 321K to 321G in the CP 47 protein of photosystem II alters the chloride requirement for growth and oxygen-evolving activity in **Synechocystis** 6803.
AUTHOR: Putnam-Evans C; Bricker T M
CORPORATE SOURCE: Department of Biology, East Carolina University, Greenville, NC 27858, USA.
SOURCE: PLANT MOLECULAR BIOLOGY, (1997 Jun) 34 (3) 455-63. Journal code: A6O. ISSN: 0167-4412.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY WEEK: 19971003
AB CP 47, a component of photosystem II (PSII) in higher plants, algae and cyanobacteria, is encoded by the psbB gene. Site-specific mutagenesis has been used to alter a portion of the psbB gene encoding the large extrinsic loop E of CP 47 in the cyanobacterium **Synechocystis** 6803. Alteration of a lysine residue occurring at position 321 to glycine produced a strain with altered PSII activity. This strain grew at wild-type rates in complete BG-11 media (480 microM chloride). However, oxygen evolution rates for this mutant in complete media were only 60% of the observed wild-type rates. Quantum yield measurements at low light intensities

indicated that the mutant had 66% of the fully functional PSII centers contained in the control strain. The mutant proved to be extremely sensitive to photoinactivation at high light intensities, exhibiting a 3-fold increase in the rate of photoinactivation. When this mutant was grown in media depleted of chloride (30 microM chloride), it lost the ability to grow photoautotrophically while the control strain exhibited a normal rate of growth. The effect of chloride depletion on the growth rate of the mutant was reversed by the addition of 480 microM bromide to the chloride-depleted BG-11 media. In the presence of glucose, the mutant and control strains grew at comparable rates in either chloride-containing or chloride-depleted media. Oxygen evolution rates for the mutant were further depressed (28% of control rates) under chloride-limiting conditions. Addition of bromide restored these rates to those observed under chloride-sufficient conditions. Measurements of the variable fluorescence yield indicated that the mutant assembled fewer functional centers in the absence of chloride. These results indicate that the mutation K321G in CP 47 affects PSII stability and/or assembly under conditions where chloride is limiting.

L5 ANSWER 16 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 97:250759 SCISEARCH
THE GENUINE ARTICLE: WP329
TITLE: Regulation of synthesis of PSI in the cyanophytes **Synechocystis** PCC6714 and Plectonema boryanum during the acclimation of the photosystem stoichiometry to the light quality
AUTHOR: Aizawa K (Reprint); Fujita Y
CORPORATE SOURCE: UNIV MARYLAND, DEPT PLANT BIOL, COLLEGE PK, MD 20742 (Reprint); NATL INST BASIC BIOL, DEPT CELL BIOL, OKAZAKI, AICHI 444, JAPAN
COUNTRY OF AUTHOR: USA; JAPAN
SOURCE: PLANT AND CELL PHYSIOLOGY, (MAR 1997) Vol. 38, No. 3, pp. 319-326.
Publisher: JAPANESE SOC PLANT PHYSIOLOGISTS, SHIMOTACHIURI OGAWA HIGASHI KAMIKYOKU, KYOTO 602, JAPAN.
ISSN: 0032-0781.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 37
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The effects of light quality on the formation of the PSI complex were examined in **Synechocystis** PCC6714 and in Plectonema boryanum. The rate of increase in levels of core polypeptides of PSI, PsaA/B, doubled upon shift from Chl a-absorbed light (PSI light) to phycobilisome-absorbed light (PSII light). The elevated rate was decreased upon the reverse shift. Half time of the

acceleration was approximately 10 min, and that of the decrease was approximately 4 min. The rate of degradation of the polypeptides was far lower than the rate of the increase under either light regime. Neither synthesis nor degradation of the PsbA and PsbC polypeptides of PSII was significantly altered by the light quality. We conclude that synthesis of the PSI complex is chromatically regulated to allow adjustments in photosystem stoichiometry. The level of mRNA for PsaA/B was not altered by the light regime. Anomalous inhibition by chloramphenicol suggested that the regulation occurs at a step(s) other than the peptide elongation step, perhaps at the initiation of the ribosome cycle or at the insertion of Chl a for the stabilization of the polypeptides. The photoreduction of protochlorophyllide (Pchlide) was compared with the synthesis of the polypeptides in a mutant of Plectonema boryanum that lacked Pchlide dark reductase (YFC1004). The results indicated that the synthesis of stable PsaA/B polypeptides was not limited by the reduction of Pchlide, although the synthesis did depend on a supply of Chl a.

L5 ANSWER 17 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 97:89225 SCISEARCH
THE GENUINE ARTICLE: WD344
TITLE: Site-directed mutagenesis of the CP 47 protein of photosystem II: Alteration of conserved charged residues which lie within lethal deletions of the large extrinsic loop E
AUTHOR: PutnamEvans C; Wu J T; Bricker T M (Reprint)
CORPORATE SOURCE: LOUISIANA STATE UNIV, DEPT MICROBIOL, BATON ROUGE, LA 70803 (Reprint); LOUISIANA STATE UNIV, DEPT MICROBIOL, BATON ROUGE, LA 70803; LOUISIANA STATE UNIV, DEPT PLANT PATHOL, BATON ROUGE, LA 70803; E CAROLINA UNIV, DEPT BIOL, GREENVILLE, NC 27858
COUNTRY OF AUTHOR: USA
SOURCE: PLANT MOLECULAR BIOLOGY, (DEC 1996) Vol. 32, No. 6, pp. 1191-1195.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0167-4412.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 21
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The intrinsic chlorophyll-protein CP 47 is a component of photosystem II which functions in both light-harvesting and oxygen evolution. The large extrinsic loop E of this protein has been shown to interact with the oxygen-evolving site. Previously, Vermaas and coworkers have produced a number of deletions within loop E which yielded mutants which were unable to grow photoautotrophically and which could not evolve oxygen at normal rates. During the course of

our site-directed mutagenesis program in **Synechocystis** 6803, we have altered all of the conserved charged residues which were present within six of these deletions. All ten of these mutants were photoautotrophic and evolved oxygen at normal rates. We speculate that the severe phenotypes of the deletion mutants observed by Vermaas and coworkers is due to large structural perturbations in the extrinsic loop E of CP 47.

L5 ANSWER 18 OF 33 MEDLINE
ACCESSION NUMBER: 97134943 MEDLINE
DOCUMENT NUMBER: 97134943
TITLE: Site-directed mutagenesis of the CP 47 protein of photosystem II: 167W in the lumenally exposed loop C is required for photosystem II assembly and stability.
AUTHOR: Wu J; Putnam-Evans C; Bricker T M
CORPORATE SOURCE: Department of Plant Biology, Louisiana State University, Baton Rouge 70803, USA.
SOURCE: PLANT MOLECULAR BIOLOGY, (1996 Nov) 32 (3) 537-42. Journal code: A6O. ISSN: 0167-4412.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
AB The intrinsic chlorophyll-protein CP 47 is a component of photosystem II which functions in both light-harvesting and oxygen evolution. Using site-directed mutagenesis we have produced the mutant W167S which lies in loop C of CP 47. This strain exhibited a 75% loss in oxygen evolution activity and grew extremely slowly in the absence of glucose. Examination of normalized oxygen evolution traces indicated that the mutant was susceptible to photoinactivation. Analysis of the variable fluorescence yield indicated that the mutant accumulated very few functional PS II reaction centers. This was confirmed by immunoblotting experiments. Interestingly, when W167S was grown in the presence of 20 microM DCMU, the mutant continued to exhibit these defects. These results indicate that tryptophan 167 in loop C of CP 47 is important for the assembly and stability of the PS II reaction center.

L5 ANSWER 19 OF 33 MEDLINE
ACCESSION NUMBER: 96070932 MEDLINE
DOCUMENT NUMBER: 96070932
TITLE: Transient accumulation of heme O (cytochrome o) in the cytoplasmic membrane of semi-anaerobic Anacystis nidulans. Evidence for oxygenase-catalyzed heme O/A transformation.
AUTHOR: Peschek G A; Alge D; Fromwald S; Mayer B
CORPORATE SOURCE: Institute of Physical Chemistry, University of

Vienna, Austria.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 17) 270 (46) 27937-41.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199603

AB Incubation of obligately photoautotrophic and aerobic cyanobacterium Anacystis nidulans (Synechococcus sp. PCC 6301) in the light in the presence of the photo-system II inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea and equilibrated with approximately 1% (v/v) O2 in N2 (10 microM O2 in solution) led to a decrease of the heme A content of isolated cytoplasmic membranes and to the appearance of heme O. The latter was not seen in membranes from fully aerated cells (> 210 microM dissolved O2). Non-covalently bound hemes extracted from the membranes were identified by reversed phase high performance liquid chromatography. Heme A and O contents of the membranes changed in a reversible fashion solely depending on the ambient oxygen regime. Both hemes A and O combine with the same **apoprotein** as suggested by immunoblotting. CO/reduced-minus-reduced optical difference spectra, photoaction spectra of CO-inhibited O2 uptake by the membranes, and pyridine hemochrome spectra pointed to either heme belonging to a functional form of the terminal oxidase. The NADH:O2 oxidoreductase reaction catalyzed by membranes from both high O2 and low O2 cells was strictly dependent on the addition of catalytic amounts of cytochrome c, fully inhibited by 1.2 microM KCN, and insensitive to 5 microM 2-n-heptyl-4-hydroxyquinoline-N-oxide. O2 uptake by the membranes was effectively catalyzed by N,N,N',N'-tetramethyl-p-phenylenediamine but not 2-methylnaphthoquinol or plastoquinol-1 as artificial substrates. Therefore we conclude that the cyanobacterial respiratory oxidase, irrespective of the type of heme in its O2-reducing center, is a cytochrome c rather than a quinol oxidase.

L5 ANSWER 20 OF 33 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 95204491 MEDLINE
DOCUMENT NUMBER: 95204491
TITLE: The role of cytochrome c-550 as studied through reverse genetics and mutant characterization in **Synechocystis** sp. PCC 6803.
AUTHOR: Shen J R; Vermaas W; Inoue Y
CORPORATE SOURCE: Solar Energy Research Group, Institute of Physical and Chemical Research (RIKEN), Saitama, Japan..
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Mar 24) 270 (12) 6901-7.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-D45178
ENTRY MONTH: 199506

AB The gene coding for cytochrome c-550 in **Synechocystis** sp. PCC 6803 was cloned based on the N-terminal sequence of the mature polypeptide. Using the most probable translation start codon, the gene is expected to code for 160 amino acid residues. This includes a cleavable N-terminal leader sequence of 25 residues. This leader sequence has an Arg-Asn-Arg sequence immediately before the cleavage site; this is characteristic for transit peptides in prokaryotes. Comparison of this sequence with the leader sequence of the photosystem II-associated extrinsic 33-kDa protein from the same cyanobacterium showed an identity of 13 out of 25 residues. These results suggest that after synthesis of the **apoprotein**, cytochrome c-550 is transported into the thylakoid lumen. Using the cloned gene, insertion and deletion mutants of **Synechocystis** sp. PCC 6803 were constructed. In the absence of cytochrome c-550, both mutants were capable of photoautotrophic growth but at a significantly reduced rate. Atrazine bindng and Western blot analysis showed that these mutants on a per-chlorophyll basis contained 53-67% of the amount of photosystem II as compared with wild type. The photosystem II-specific oxygen-evolving activity at saturating light intensity was reduced to about 40% of that in the wild type strain. Taken together, these results indicate that the cytochrome c-550 is transported into the thylakoid lumen and contributes to optimal functional stability of photosystem II in cyanobacteria. This supports our biochemical evidence that cytochrome c-550 is associated with the lumenal side of photosystem II as one of the extrinsic proteins enhancing oxygen evolution (Shen, J.-R., Ikeuchi, M., and Inoue, Y. (1992) FEBS Lett. 301, 145-149; Shen, J.-R., and Inoue, Y. (1993) Biochemistry 32, 1825-1832). Based on these results, the gene for cytochrome c-550 was named psbV. The possible evolutionary relationship among extrinsic proteins of the photosystem II donor side is discussed.

L5 ANSWER 21 OF 33 MEDLINE
ACCESSION NUMBER: 95275883 MEDLINE
DOCUMENT NUMBER: 95275883
TITLE: Involvement of the CP47 protein in stabilization and photoactivation of a functional water-oxidizing complex in the cyanobacterium **Synechocystis** sp. PCC 6803.
AUTHOR: Gleiter H M; Haag E; Shen J R; Eaton-Rye J J; Seeliger A G; Inoue Y; Vermaas W F; Renger G
CORPORATE SOURCE: Max-Volmer-Institute for Physical and Biophysical Chemistry, Technical University Berlin, Germany..
SOURCE: BIOCHEMISTRY, (1995 May 23) 34 (20) 6847-56.
Searcher : Shears 308-4994

Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509

AB Oscillation patterns of the oxygen yield per flash induced by a train of single-turnover flashes were measured as a function of dark incubation and different pre-illumination conditions in several autotrophic mutant strains of **Synechocystis** sp. PCC 6803 carrying short deletions within the large, lumen-exposed hydrophilic region (loop E) of the chlorophyll a-binding photosystem II protein CP47. A physiological and biochemical characterization of these mutant strains has been presented previously [Eaton-Rye, J. J., & Vermaas, W. F. J. (1991) Plant Mol. Biol. 17, 1165-1177; Haag, E., Eaton-Rye, J. J., Renger, G., & Vermaas, W. F. J. (1993) Biochemistry 32, 4444-4454], and some functional properties were described recently [Gleiter, H. M., Haag, E., Shen, J.-R., Eaton-Rye, J. J., Inoue, Y., Vermaas, W. F. J., & Renger, G. (1994) Biochemistry 33, 12063-12071]. The present study shows that in several mutants the water-oxidizing complex (WOC) became inactivated during prolonged dark incubation, whereas the WOC of the wild-type strain remained active. The rate and extent of the inactivation in the mutants depend on the domain of loop E, where 3-8 amino acid residues were deleted. The most pronounced effects are observed in mutants delta(A373-D380) and delta(R384-V392). A competent WOC can be restored from the fully inactivated state by illumination with short saturating flashes. The number of flashes required for this process strongly depends on the site at which a deletion has been introduced into loop E. Again, the most prominent effects were found in mutants delta(A373-D380) and delta(R384-V392). Interestingly, the number of flashes required for activation was reduced by more than an order of magnitude in both mutants by the addition of 10 mM CaCl2 to the cell suspension. On the basis of a model for photoactivation proposed by Tamura and Cheniae (1987) [Biochim. Biophys. Acta 890, 179-194], a scheme is presented for the processes of dark inactivation and photoactivation in these mutants. The results presented here corroborate an important role of the large hydrophilic domain (loop E) of CP47 in a functional and stable WOC.

L5 ANSWER 22 OF 33 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 96340947 MEDLINE
DOCUMENT NUMBER: 96340947
TITLE: Promiscuity of heme groups in the cyanobacterial cytochrome-C oxidase.
AUTHOR: Auer G; Mayer B; Wastyn M; Fromwald S; Eghbalzad K; Alge D; Peschek G A
CORPORATE SOURCE: Institute of Physical Chemistry, University of Vienna, Austria.

SOURCE: BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, (1995 Dec) 37 (6) 1173-85.
Journal code: BOD. ISSN: 1069-8302.
PUB. COUNTRY: Australia
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612

AB The cyanobacteria Nostoc sp. strain Mac, Anabaena 7937, **Synechocystis** 6803, and Anacystis nidulans (Synechococcus 6301) were grown and incubated in the light under three different oxygen regimes: Phase-A cells were harvested from photoautotrophically growing cultures at a cell density of 2.8-3.2 microliter packed cell mass/ml and an oxygen concentration of approx. 350 microM (corresponding to > 150% air saturation). Phase-B cells were harvested 24 hrs after 20 microM 3-(3,4-dichlorophyl)-1,1-dimethylurea had been added to the culture and gassing switched to 1% oxygen (< 10 microM). Phase-C cells originated from phase-B cells after 12 hrs of gassing the illuminated, yet non-growing cultures with air (21% oxygen or 200-220 microM in the medium). Cytoplasmic membranes were isolated and purified from each of the three cell types. Non-covalently bound hemes were extracted and identified by reversed-phase high performance liquid chromatography. Besides ubiquitous heme B only heme A was detected in phase-A membranes while phase-B and phase-C membranes contained both hemes A and O proportions of which depended on the oxygen status of the cells. CO/difference spectra, photo-action spectra of CO-inhibited oxygen uptake, and polarographic determination of oxygen-affinities clearly showed that both hemes A and O were part of a functional form of cytochrome-c oxidase which, however, exhibited a single subunit-I **apoprotein** as verified by immunoblotting. Also electron transport characteristics did not give evidence for a quinol or any other alternate oxidase functioning in cyanobacteria.

L5 ANSWER 23 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 95:427389 SCISEARCH
THE GENUINE ARTICLE: RD562

TITLE: THE ACCUMULATION OF PROTOCHLOROPHYLLIDE IN CELLS OF **SYNECHOCYSTIS** PCC-6714 WITH A LOW PSI PSII STOICHIOMETRY

AUTHOR: FUJITA Y (Reprint); MURAKAMI A; AIZAWA K
CORPORATE SOURCE: TSURUKAWA 5-15-11, MACHIDA, TOKYO 195, JAPAN (Reprint); NATL INST BASIC BIOL, DEPT CELL BIOL, OKAZAKI, AICHI 444, JAPAN
COUNTRY OF AUTHOR: JAPAN

SOURCE: PLANT AND CELL PHYSIOLOGY, (JUN 1995) Vol. 36, No. 4, pp. 575-582.
ISSN: 0032-0781.
DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 24
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Changes in intracellular levels of Chl a precursors were examined in relation to changes in the PSI/PSII stoichiometry in the cyanophyte **Synechocystis** PCC 6714. Protochlorophyllide (Pchlide) accumulated markedly in cells with a low PSI/PSII stoichiometry grown under light that is absorbed by Chl a (PSI light) whereas no accumulation occurred in cells with a high PSI/PSII stoichiometry grown under light absorbed by phycobilisomes (PSII light). Levels of Pchlide in cells grown under PSI light decreased rapidly upon a shift to PSII light. The rapid decrease in Pchlide accompanied a transient increase in chlorophyllide a, indicating that reduction of Pchlide was enhanced by shift to PSII light. The action spectrum indicated that the Pchlide decrease upon the shift to PSII light depended on excitation of Pchlide, suggesting that the accumulation of Pchllide was due to limited excitation of Pchlide, so that Pchlide photoreduction, under PSI light. However, comparison of levels of Pchlide and the photosystem complexes in wild-type Plectonema boryanum with those in a mutant that lacked the dark Pchlide reductase (YFC 1004) indicated that dark reduction compensated for the limited photoreduction under PSI light. Similar compensation by dark reduction was confirmed with **Synechocystis** PCC 6714. In cultures of **Synechocystis** under conditions where Pchlide could not be photoreduced, accumulation of Pchlide and low PSI/PSII stoichiometry occurred only when cells were illuminated with light that preferentially excited PSI. The results indicate that the low PSI/PSII stoichiometry in cells grown under PSI light is not a result of inefficient synthesis of Chl a with a reduced rate of Pchlide photoreduction. They suggest further that accumulation of Pchlide under PSI light results from retardation of the Chl a synthesis due to suppression of PSI synthesis.

L5 ANSWER 24 OF 33 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 95402212 MEDLINE
DOCUMENT NUMBER: 95402212
TITLE: Occurrence of heme O in photoheterotrophically growing, semi-anaerobic cyanobacterium **Synechocystis** sp. PCC6803.
AUTHOR: Peschek G A; Wastyn M; Fromwald S; Mayer B
CORPORATE SOURCE: Institute of Physical Chemistry, University of Vienna, Austria..
SOURCE: FEBS LETTERS, (1995 Sep 4) 371 (2) 89-93.
Journal code: EUH. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199512
AB Extraction and identification of the non-covalently bound heme groups from crude membrane preparations of photoheterotrophically grown **Synechocystis** sp. PCC 6803 by reversed phase high performance liquid chromatography and optical spectrophotometry led to the detection of heme O in addition to hemes B and A which latter was to be expected from the known presence of aa3-type cytochrome oxidase in cyanobacteria. In fully aerated cells (245 microM dissolved O2 in the medium) besides heme B only heme A was found while in low-oxygen cells (< 10 microM dissolved O2) heme O was present at a concentration even higher than that of heme A. Given the possible role of heme O as a biosynthetic intermediate between heme B and heme A, together with generally much higher Km values of 5-50 microM O2 for oxygenase as compared to Km values of 40-70 nM O2 for typical cytochrome-c oxidase, our findings may permit the conclusion that the conversion of heme O to heme A is an obligately oxygen-requiring process catalyzed by some oxygenase directly introducing oxygen from O2 into the 8-methyl group of heme O. At the same time thus the occurrence of heme O (cytochrome o) in cyanobacteria does of course not imply the existence of an 'alternative oxidase' since according to the well-known 'promiscuity of heme groups' both hemes O and A are likely to combine with one and the same **apoprotein**.

L5 ANSWER 25 OF 33 MEDLINE
ACCESSION NUMBER: 94355301 MEDLINE
DOCUMENT NUMBER: 94355301
TITLE: Site-directed mutagenesis of the CP47 protein of photosystem II: alteration of the basic residue 448R to 448G prevents the assembly of functional photosystem II centers under chloride-limiting conditions.
AUTHOR: Putnam-Evans C; Bricker T M
CORPORATE SOURCE: Department of Biology, East Carolina University, Greenville, North Carolina 27858..
SOURCE: BIOCHEMISTRY, (1994 Sep 6) 33 (35) 10770-6.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199412
AB The psbB gene encodes the intrinsic chlorophyll protein CP47 (CPa-1), a component of photosystem II in higher plants, algae, and cyanobacteria. Oligonucleotide-directed mutagenesis has been used to introduce mutations into a segment of the psbB gene which encodes the large extrinsic loop E of CP47 in the cyanobacterium **Synechocystis** sp. PCC 6803. One mutation, R448G, produced a

strain with impaired photosystem II activity. When grown in standard BG-11 media (480 microM chloride), this strain grew photoautotrophically at about 50% the rate of control strains and exhibited 63% of the control photosystem II activity. Quantum yield measurement at low light intensities indicated that this mutant had 55% of the fully functional photosystem II centers contained in control strains of **Synechocystis**. Upon exposure to high light intensities, the mutant strain exhibited a 2.2-fold increase in the rate of photoinactivation. When grown in BG-11 which was depleted in chloride (20 microM chloride), the mutant strain exhibited dramatically altered characteristics. Little or no growth was observed in the mutant while the control strains grew at nearly normal rates. Growth rates of the mutant strain could be restored by the addition of 480 microM bromide to the chloride-deficient BG-11 media. In the presence of glucose, the mutant and control strains grew at comparable rates under either chloride-sufficient or chloride-limiting conditions. Analysis of the mutant cell line grown in the absence of chloride and in the presence of glucose indicated that it exhibited essentially no capacity for oxygen evolution.(ABSTRACT TRUNCATED AT 250 WORDS)

L5 ANSWER 26 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 94:357752 SCISEARCH
THE GENUINE ARTICLE: NP931
TITLE: MOLECULAR TOPOLOGY OF THE PHOTOSYSTEM-II CHLOROPHYLL-ALPHA BINDING-PROTEIN, CP-43 - TOPOLOGY OF A THYLAKOID MEMBRANE-PROTEIN
AUTHOR: SAYRE R T (Reprint); WROBELBOERNER E A
CORPORATE SOURCE: OHIO STATE UNIV, DEPT PLANT BIOL, 2021 COFFEY RD, COLUMBUS, OH, 43210 (Reprint); OHIO STATE UNIV, DEPT BIOCHEM, COLUMBUS, OH, 43210
COUNTRY OF AUTHOR: USA
SOURCE: PHOTOSYNTHESIS RESEARCH, (APR 1994) Vol. 40, No. 1, pp. 11-19.
ISSN: 0166-8595.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 23
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have used antibodies generated against synthetic peptides to determine the topology of the 43 kD chlorophyll a binding protein (CP 43) of Photosystem II. Based on the pattern of proteolytic fragments detected (on western blots) by peptide specific antibodies, a six transmembrane span topological model, with the amino and carboxyl termini located on the stromal membrane surface, is predicted. This structure is similar to that predicted for CP 47, a PS II chlorophyll a binding protein (Bricker T (1990) Photosynth Res 24: 1-13). The model is discussed in reference to the possible

location of chlorophyll binding sites.

L5 ANSWER 27 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 92:692477 SCISEARCH
THE GENUINE ARTICLE: JZ523
TITLE: SITE-DIRECTED MUTAGENESIS OF THE CPA-1 PROTEIN OF PHOTOSYSTEM-II - ALTERATION OF THE BASIC RESIDUE PAIR (384,385)R TO (384,385)G LEADS TO A DEFECT ASSOCIATED WITH THE OXYGEN-EVOLVING COMPLEX
AUTHOR: PUTNAMEVANS C; BRICKER T M (Reprint)
CORPORATE SOURCE: LOUISIANA STATE UNIV, DEPT BOT, BATON ROUGE, LA, 70803
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (24 NOV 1992) Vol. 31, No. 46, pp. 11482-11488.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The psbB gene encodes the intrinsic chlorophyll-a binding protein CPa-1 (CP-47), a component of photosystem II in higher plants, algae, and cyanobacteria. Oligonucleotide-directed mutagenesis was used to introduce mutations into a segment of the psbB gene encoding the large extrinsic loop region of CPa-1 in the cyanobacterium **Synechocystis** sp. PCC 6803. Altered psbB genes were introduced into a mutant recipient strain (DEL-1) of **Synechocystis** in which the genomic psbB gene had been partially deleted. Initial target sites for mutagenesis were absolutely conserved basic residue pairs occurring within the large extrinsic loop. One mutation, RR384385GG, produced a strain with impaired photosystem II activity. This strain exhibited growth characteristics comparable to controls. However, at saturating light intensities this mutant strain evolved oxygen at only 50% of the rate of the control strains. Quantum yield measurements at low light intensities indicated that the mutant had 30% fewer fully functional photosystem II centers than do control strains of **Synechocystis**. Immunological analysis of a number of photosystem II protein components indicated that the mutant accumulates normal quantities of photosystem II proteins and that the ratio of photosystem II to photosystem I proteins is comparable to that found in control strains. Upon exposure to high light intensities the mutant cells exhibited a markedly increased susceptibility to photoinactivation. However, Tris-treated thylakoid membranes from both the mutant and wild-type exhibited comparable rates of photoinactivation. Thylakoid membranes isolated from RR384385GG exhibited only 15% of the H2O to 2,6-dichlorophenolindophenol electron transport rate observed in

wild-type strains. The 1,5-diphenylcarbazide to 2,6-dichlorophenolindophenol electron transport rates of Tris-treated thylakoids from the mutant, however, were comparable to control rates. These results suggest that alteration of this basic residue pair leads to a defect associated with the oxygen-evolving complex of photosystem II.

L5 ANSWER 28 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 92:402646 SCISEARCH
THE GENUINE ARTICLE: JB495
TITLE: CHANGES IN COMPOSITION OF MEMBRANE-PROTEINS ACCOMPANYING THE REGULATION OF PS-I/PS-II STOICHIOMETRY OBSERVED WITH **SYNECHOCYSTIS** PCC-6803
AUTHOR: AIZAWA K; SHIMIZU T; HIYAMA T; SATOH K; NAKAMURA Y; FUJITA Y (Reprint)
CORPORATE SOURCE: NATL INST BASIC BIOL, OKAZAKI, AICHI 444, JAPAN; SAITAMA UNIV, FAC SCI, DEPT BIOCHEM, URAWA, SAITAMA 338, JAPAN; TOYOTA MOTOR CO, BIORES LAB, TOYOTA, AICHI 471, JAPAN; OKAYAMA UNIV, FAC SCI, DEPT BIOL, OKAYAMA 700, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: PHOTOSYNTHESIS RESEARCH, (MAY 1992) Vol. 32, No. 2, pp. 131-138.
ISSN: 0166-8595.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Changes in composition of membrane proteins in **Synechocystis** PCC 6803 induced by the shift of light regime for photosynthetic growth were studied in relation to the regulation of PS I/PS II stoichiometry. Special attention was paid to the changes in abundance of proteins of PS I and PS II complexes. Composition was examined using a LDS-PAGE and a quantitative enzyme immunoassay. Abundance of PsaA/B polypeptides and the PsaC polypeptide of the PS I complex, on a per cell basis, increased under the light regime exciting preferentially PS II and decreased under the light regime exciting mainly PS I. Similar changes were observed with polypeptides of 18.5, 10 and 8.5 kDa. The abundance of other proteins associated with membranes, including PsbA polypeptide of the PS II complex, was fairly constant irrespective of light regime. These results are consistent with our previous observations with other strains of cyanophytes (Anabaena variabilis M2 and **Synechocystis** PCC 6714) that PS I is the variable component in changes in PS I/PS II stoichiometry in response to changing light regimes for photosynthesis.

Searcher : Shears 308-4994

L5 ANSWER 29 OF 33 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 92007780 MEDLINE
DOCUMENT NUMBER: 92007780
TITLE: Targeted genetic inactivation of the photosystem I reaction center in the cyanobacterium **Synechocystis** sp. PCC 6803.
AUTHOR: Smart L B; Anderson S L; McIntosh L
CORPORATE SOURCE: DOE-Plant Research Laboratory, Michigan State University, East Lansing 48824..
SOURCE: EMBO JOURNAL, (1991 Nov) 10 (11) 3289-96.
Journal code: EMB. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199201
AB We describe the first complete segregation of a targeted inactivation of psaA encoding one of the P700-chlorophyll a **apoproteins** of photosystem (PS) I. A kanamycin resistance gene was used to interrupt the psaA gene in the unicellular cyanobacterium **Synechocystis** sp. PCC 6803. Selection of a fully segregated mutant, ADK9, was performed under light-activated heterotrophic growth (LAHG) conditions; complete darkness except for 5 min of light every 24 h and 5 mM glucose. Under these conditions, wild-type cells showed a 4-fold decrease in chlorophyll (chl) per cell, primarily due to a decrease of PS I reaction centers. Evidence for the absence of PS I in ADK9 includes: the lack of EPR (electron paramagnetic resonance) signal I, from P700+; undetectable P700-**apoprotein**; greatly reduced whole-chain photosynthesis rates; and greatly reduced chl per cell, resulting in a turquoise blue phenotype. The PS I peripheral proteins PSA-C and PSA-D were not detected in this mutant. ADK9 does assemble near wild-type levels of functional PS II per cell, evidenced by: EPR signal II from YD+; high rates of oxygen evolution with 2,6-dichloro-p-benzoquinone (DCBQ), an electron acceptor from PS II; and accumulation of D1, a PS II core polypeptide. The success of this transformation indicates that this cyanobacterium may be utilized for site-directed mutagenesis of the PS I core.

L5 ANSWER 30 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 91:602484 SCISEARCH
THE GENUINE ARTICLE: GM462
TITLE: EXPRESSION OF PHOTOSYNTHESIS GENES IN THE CYANOBACTERIUM **SYNECHOCYSTIS** SP PCC-6803 - PSAA-PSAB AND PSBA TRANSCRIPTS ACCUMULATE IN DARK-GROWN CELLS
AUTHOR: SMART L B; MCINTOSH L (Reprint)
CORPORATE SOURCE: MICHIGAN STATE UNIV, US DOE, PLANT RES LAB, E LANSING, MI, 48824; MICHIGAN STATE UNIV, DEPT

BIOCHEM, E LANSING, MI, 48824; MICHIGAN STATE UNIV, US DOE, GENET PROGRAM, E LANSING, MI, 48824
COUNTRY OF AUTHOR: USA
SOURCE: PLANT MOLECULAR BIOLOGY, (1991) Vol. 17, No. 5, pp. 959-971.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have cloned and sequenced the psaA and psaB genes from the unicellular cyanobacterium **Synechocystis** sp. PCC 6803. These genes are arranged in tandem, are co-transcribed, and are highly homologous to the psaA and psab genes previously characterized. RNA was isolated from light-grown cells, from cells put in total darkness with and without glucose, and from cells grown under light-activated heterotrophic growth (LAHG) conditions. Quantitation of hybridization to northern blots revealed only a slight decrease in the accumulation of the psaA-psaB transcript in cells grown in complete darkness with glucose and in LAHG cells, relative to light-grown cells. Accumulation of the psbA transcript steadily declines through dark incubation, with a steady-state level in LAHG cells 28% of that in light-grown cells. Transcripts from psbD, psaD, and rbcLS accumulate in cells grown in complete darkness and in LAHG cells to approximately the same levels as in light-grown cells. Photosynthesis gene transcripts in cells grown in the dark without glucose were detected, but were highly degraded. Our data prove that transcripts from photosynthesis genes do accumulate in dark-grown **Synechocystis** 6803, which may allow for synthesis and assembly of photosystem (PS) I and PS II in the dark.

L5 ANSWER 31 OF 33 SCISEARCH COPYRIGHT 1999 ISI (R)
ACCESSION NUMBER: 91:504625 SCISEARCH
THE GENUINE ARTICLE: GE273
TITLE: CHARACTERIZATION OF GENES THAT ENCODE SUBUNITS OF CUCUMBER PS-I COMPLEX BY N-TERMINAL SEQUENCING
AUTHOR: IWASAKI Y; ISHIKAWA H; HIBINO T; TAKABE T (Reprint)
CORPORATE SOURCE: MEIJO UNIV, FAC SCI & TECHNOL, DEPT CHEM, 1-501 SHIOGAMAGUCHI, TENPAKU KU, NAGOYA, AICHI 468, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1991) Vol. 1059, No. 2, pp. 141-148.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 41

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB N-terminal amino acid sequencing was carried out to characterize the genes of the cucumber PS I complex (PSI-100) that contains eight

polypeptides and catalyzes the light-dependent transfer of electrons from plastocyanin to ferredoxin. The genes of all subunits except the 17.5 kDa polypeptide in PSI-100 have been identified. These are psaA/psaB (65/63 kDa), psaD (20 kDa), psaE (19.5 kDa), psaF (18.5 kDa), psaH (7.6 kDa), and psaC (5.8 kDa). The 17.5 kDa polypeptide is a new protein and is designated tentatively as the gene product of psaM. N-terminal amino-acid sequencing indicated the presence of two polypeptides in the 7.6 kDa band. One of these is the gene product of psaH and is essential for the activity of the PS I complex, and the other one is as yet unrecognized and largely depleted in the PSI-100 complex. Gene products of psaG, psaI, and psaK, which have been proposed as the components of PS I complex, are not involved in the PSI-100 complex, but are involved in the PS I complex (PSI-200), which contains 120 chlorophyll per reaction center chlorophyll (P700) and light-harvesting chlorophyll a/b protein complexes. Three polypeptides (26, 23 and 22.5 kDa) are not involved in the PSI-100 and are assigned as the **apo-protein** of light-harvesting chlorophyll a/b protein complexes.

L5 ANSWER 32 OF 33 LIFESCI COPYRIGHT 1999 CSA
ACCESSION NUMBER: 89:88545 LIFESCI
TITLE: Specific bleaching of phycobiliproteins from cyanobacteria and red algae at high temperature in vivo.
AUTHOR: Zhao, Jin-Dong; Brand, J.J.
CORPORATE SOURCE: Dep. Bot., Univ. Texas, Austin, TX 78713, USA
SOURCE: ARCH. MICROBIOL., (1989) vol. 152, no. 5, pp. 447-452

DOCUMENT TYPE: Journal
FILE SEGMENT: K; Q4
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Exposure of blue-green or red algal cells to temperatures > 60-65 degree C for several minutes resulted in bleaching of all phycobilin absorption in the visible range, with virtually no alteration in chlorophyll or carotenoid absorption. Difference spectra of non-bleached vs bleached cells appeared identical to absorption spectra of purified phycobilisomes isolated from the same cell culture in high phosphate medium. All phycobilin chromophores were bleached at approximately the same rate during heating. There were no changes in apparent molecular weights or relative amounts of the phycobilisome **apoproteins** during chromophore bleaching. Phycobilisomes in cell extracts from Anacystis nidulans resisted bleaching when suspended in medium of high phosphate concentration, but were bleached at 60-65 degree C within a few minutes when placed in diluted medium. The results indicate that phycobilisomes in vivo are stabilized by a mechanism other than high osmotic and ionic strength.

L5 ANSWER 33 OF 33 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1989:386346 BIOSIS
DOCUMENT NUMBER: BA88:66936
TITLE: VISUALIZATION OF ANTIBODY BINDING TO THE PHOTOSYNTHETIC MEMBRANE THE TRANSMEMBRANE ORIENTATION OF CYTOCHROME B-559.
AUTHOR(S): VALLON O; TAE G-S; CRAMER W A; SIMPSON D; HOYER-HANSEN G; BOGORAD L
CORPORATE SOURCE: LAB. MICROSCOPIE ELECTRONIQUE, INST. JACQUES MONOD, CNRS, UNIV. PARIS VII, 2 PLACE JUSSIEU, T 43, 75005 PARIS, FR.
SOURCE: BIOCHIM BIOPHYS ACTA, (1989) 975 (1), 132-141. CODEN: BBACAQ. ISSN: 0006-3002.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB We have used immuno-gold labeling and electron microscopy to study the topography of thlakoid membrane polypeptides. Thylakoid vesicles formed by passage through a French press were adsorbed onto a plastic film supported by an electron microscope grid and processed for single or double immuno-gold labeling. After shadowing with platinum, the inside-out and right-side-out vesicles were identified by their distinctive morphologies. Right-side-out vesicles were labeled by a monoclonal antibody recognizing an epitope located in the trypsin-cleaved, N-terminal portion of the LHC II **apoprotein**, and by an antibody to CF1. A monoclonal antibody to the .alpha.-subunit of cytochrome .beta.-559 reacted with a synthetic tridecapeptide corresponding to the C-terminal portion of the polypeptide. Both this antibody and a polyclonal antibody to the synthetic peptide labeled inside-out vesicles exclusively, indicating that the polypeptide C-terminus was exposed on the lumenal (exoplasmic) surface of the membrane.

=> d his l6-; d 1-14 ibib abs

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, BIOTECHDS, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT' ENTERED AT 14:18:22 ON 13 SEP 1999)

- Author

L6 448 S LAGARIAS J?/AU
L7 55 S L6 AND (APOPROTEIN OR APO PROTEIN)
L8 14 DUP REM L7 (41 DUPLICATES REMOVED)

L8 ANSWER 1 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
ACCESSION NUMBER: 1998:112490 CAPLUS
DOCUMENT NUMBER: 128:190158
TITLE: Phytofluors as fluorescent labels
INVENTOR(S): **Lagarias, John Clark**; Murphy, John Thomas
PATENT ASSIGNEE(S): Regents of the University of California, USA;

	Lagarias, John Clark; Murphy, John Thomas
SOURCE:	PCT Int. Appl., 87 pp.
	CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9805944	A1	19980212	WO 1997-US13529	19970801
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 916085	A1	19990519	EP 1997-936339	19970801
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:	US 1996-23217 19960802
	WO 1997-US13529 19970801

AB This invention provides new fluorescent mols. useful for detection of target entities. In particular, it relates to fluorescent adducts comprising an **apoprotein** and a bilin.

L8 ANSWER 2 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2

ACCESSION NUMBER:	1997:772086 CAPLUS
DOCUMENT NUMBER:	128:112486
TITLE:	The phytofluors: a new class of fluorescent protein probes
AUTHOR(S):	Murphy, John T.; **Lagarias, J. Clark**
CORPORATE SOURCE:	Section of Molecular and Cellular Biology, University of California, Davis, CA, 9561 6, USA
SOURCE:	Curr. Biol. (1997), 7(11), 870-876
	CODEN: CUBLE2; ISSN: 0960-9822
PUBLISHER:	Current Biology Ltd.
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB Biol. compatible fluorescent protein probes, particularly the self-assembling green fluorescent protein (GFP) from the jellyfish Aequorea victoria, have revolutionized research in cell, mol. and developmental biol. because they allow visualization of biochem. events in living cells. Addnl. fluorescent proteins that could be reconstituted in vivo while extending the useful wavelength range towards the orange and red regions of the light spectrum would increase the range of applications currently available with fluorescent protein probes. Intensely orange fluorescent adducts, which we designate phytofluors, are spontaneously formed upon incubation of recombinant plant phytochrome **apoproteins** with phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin chromophore. Phytofluors have large molar absorption

coeffs., fluorescence quantum yields greater than 0.7, excellent photostability, stability over a wide range of pH, and can be reconstituted in living plant cells. The phytofluors constitute a new class of fluorophore that can potentially be produced upon bilin uptake by any living cell expressing an apophytochrome cDNA. Mutagenesis of the phytochrome **apoprotein** and/or alteration of the linear tetrapyrrole precursor by chem. synthesis are expected to afford new phytofluors with fluorescence excitation and emission spectra spanning the visible to near-IR light spectrum.

L8 ANSWER 3 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
ACCESSION NUMBER: 1996:524194 CAPLUS
DOCUMENT NUMBER: 125:190274
TITLE: The methylotrophic yeast Pichia pastoris synthesizes a functionally active chromophore precursor of the plant photoreceptor phytochrome
AUTHOR(S): Wu, Shu-Hsing; **Lagarias, J. Clark**
CORPORATE SOURCE: Section Molecular Cellular Biology, Univ. California, Davis, CA, 95616, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1996), 93(17), 8989-8994
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Induction of the expression of an algal phytochrome cDNA in the methylotrophic yeast Pichia pastoris led to time-dependent formation of photoactive holophytochrome without the addn. of exogenous bilins. Both in vivo and in vitro difference spectra of this photochromic species are very similar to those of higher plant phytochrome A, supporting the conclusion that this species possesses a phytochromobilin prosthetic group. Zinc blot analyses confirm that a bilin chromophore is covalently bound to the algal phytochrome **apoprotein**. The hypothesis that P. pastoris contains phytochromobilin synthase, the enzyme that converts biliverdin IX.alpha. to phytochromobilin, was also addressed in this study. Sol. exts. from P. pastoris were able to convert biliverdin to a bilin pigment, which produced a native difference spectrum upon assembly with oat apophytochrome A. HPLC analyses confirm that biliverdin is converted to both 3E- and 3Z-isomers of phytochromobilin. These investigations demonstrate that the ability to synthesize phytochromobilin is not restricted to photosynthetic organisms and support the hypothesis of a more widespread distribution of the phytochrome photoreceptor.

L8 ANSWER 4 OF 14 MEDLINE
ACCESSION NUMBER: 95315179 MEDLINE
DOCUMENT NUMBER: 95315179
TITLE: Continuous fluorescence assay of phytochrome assembly in vitro.

AUTHOR: Li L; Murphy J T; **Lagarias J C**
CORPORATE SOURCE: Section of Molecular and Cellular Biology, University of California, Davis 95616, USA.
CONTRACT NUMBER: 5 T32 GM07377-17 (NIGMS)
SOURCE: BIOCHEMISTRY, (1995 Jun 20) 34 (24) 7923-30.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
AB Incubation of recombinant apophytochrome with the phycobiliprotein chromophore precursor phycoerythrobilin produces a covalent adduct that exhibits a fluorescence excitation maximum at 576 nm and an emission maximum at 586 nm. Using these fluorescence parameters, we have developed a kinetic assay for quantitative analysis of the assembly of the plant photoreceptor phytochrome in real time. Kinetic measurements performed with different phycoerythrobilin concentrations confirm that bilin attachment to apophytochrome involves two steps, an initial formation of a reversible non-covalent complex followed by thioether bond formation. The kinetic constants for both steps of phycoerythrobilin attachment to apophytochrome were estimated with this assay. Methodology for determining the kinetic constants for the assembly of both the natural phytochrome chromophore precursor, phytochromobilin, and the analog phycocyanobilin is also described. Since the latter two bilins yield covalent, nonfluorescent adducts with apophytochrome, their co-incubation with phycoerythrobilin reduces the rate of formation of the fluorescent phycoerythrobilin adduct in an irreversible, competitive manner. Competition experiments were also performed with biliverdin, a structurally related bilin which does not form a covalent adduct with apophytochrome. Such measurements show that biliverdin reversibly binds to apophytochrome with a submicromolar binding constant, an affinity which is very similar to that of phytochromobilin. The utility of this fluorescence assay for identification of novel inhibitors of phytochrome assembly and for characterization of the structural features of both bilin and apophytochrome necessary for photoreceptor assembly is discussed.

L8 ANSWER 5 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4
ACCESSION NUMBER: 1995:284844 CAPLUS
DOCUMENT NUMBER: 122:73314
TITLE: Phytochrome assembly in living cells of the yeast Saccharomyces cerevisiae
AUTHOR(S): Li, Liming; **Lagarias, J. Clark**
CORPORATE SOURCE: Sect. Molec. Cellular Biol., Univ. California, Davis, CA, 95616, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1994), 91(26), 12535-9
Searcher : Shears 308-4994

CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biol. activity of the plant photoreceptor phytochrome requires the specific assocn. of a linear tetrapyrrole prosthetic group with a large **apoprotein**. As an initial step to develop an in vivo assay system for structure-function anal. of the phytochrome photoreceptor, we undertook expts. to reconstitute holophytochrome in the yeast Saccharomyces cerevisiae. Here we show that yeast cells expressing recombinant oat apophytochrome A can take up exogenous linear tetrapyrroles, and, in a time-dependent manner, these pigments combine with the **apoprotein** to form photoactive holophytochrome in situ. Cell viability measurements indicate that holophytochrome assembly occurs in living cells. Unlike phytochrome A in higher plant tissue, which is rapidly degraded upon photoactivation, the reconstituted photoreceptor appears to be light stable in yeast. Reconstitution of photoactive phytochrome in yeast cells should enable us to exploit the power of yeast genetics for structure-function dissection of this important plant photoreceptor.

L8 ANSWER 6 OF 14 MEDLINE
ACCESSION NUMBER: 94075284 MEDLINE
DOCUMENT NUMBER: 94075284
TITLE: Inactivation of phytochrome- and phycobiliprotein-chromophore precursors by rat liver biliverdin reductase [published erratum appears in J Biol Chem 1994 Apr 8;269(14):10965].
AUTHOR: Terry M J; Maines M D; **Lagarias J C**
CORPORATE SOURCE: Section of Molecular and Cellular Biology, University of California, Davis 95616..
CONTRACT NUMBER: ES04066 (NIEHS)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Dec 15) 268 (35) 26099-106.
Journal code: HIV. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199403
AB The phytochrome chromophore precursor, 3E-phytochromobilin, and the phycobiliprotein chromophore precursors, 3E-phycocyanobilin and 3E-phycoerythrobilin, are enzymatically converted to novel rubinoid products by purified rat liver biliverdin reductase. Phytochromobilin and phycocyanobilin are particularly good substrates for biliverdin reductase with Km and Vmax values very similar to those of the natural substrate, biliverdin IX alpha. Phycoerythrobilin is the least preferred of the three bilin substrates. 1H NMR spectroscopy of phycocyanorubin, the product of

phycocyanobilin catalysis by biliverdin reductase, and comparison of absorption spectra of all three rubinoid products reveal that the C10 methine bridge is selectively reduced by biliverdin reductase without altering the A-ring ethylidene substituent. In vitro phytochrome assembly experiments demonstrate that the phytorubin products do not form photoactive adducts with recombinant apophytochrome. These results suggest that ectopic expression of biliverdin reductase in plants will prevent assembly of the functional photoreceptor and thus will potentially alter light-mediated plant growth and development.

L8 ANSWER 7 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 5
ACCESSION NUMBER: 1993:645494 CAPLUS
DOCUMENT NUMBER: 119:245494
TITLE: Biosynthesis of the plant photoreceptor phytochrome
AUTHOR(S): Terry, Matthew J.; Wahleithner, Jill A.; **Lagarias, J. Clark**
CORPORATE SOURCE: Sect. Mol. Cell. Biol., Univ. California, Davis, CA, 95616, USA
SOURCE: Arch. Biochem. Biophys. (1993), 306(1), 1-15
CODEN: ABBIA4; ISSN: 0003-9861
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 159 refs. Holophytochrome biosynthesis requires the convergence of 2 biochem. pathways, one for the **apoprotein** and another for the linear tetrapyrrole prosthetic group. Major advances have been made in understanding the biosynthesis of the phytochrome chromophore precursor phytochromobilin, its assembly with the phytochrome **apoprotein**, and the mol. processes that contribute to the regulation of holophytochrome synthesis.

L8 ANSWER 8 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6
ACCESSION NUMBER: 1992:528352 CAPLUS
DOCUMENT NUMBER: 117:128352
TITLE: Phytochrome assembly. Defining chromophore structural requirements for covalent attachment and photoreversibility
AUTHOR(S): Li, Liming; **Lagarias, J. Clark**
CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: J. Biol. Chem. (1992), 267(27), 19204-10
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Assembly of holophytochrome in the plant cell requires covalent attachment of the linear tetrapyrrole chromophore precursor, phytochromobilin, to a unique cysteine in the nascent **apoprotein**. Chromophore analogs were compared with the

natural chromophore precursor for their ability to attach covalently to recombinant oat apophytochrome and to form photoactive holoproteins. Ethylidene-contg. analogs readily form covalent adducts with apophytochrome, whereas chromophores lacking this double bond are poor substrates for attachment. Kinetic measurements establish that although the chromophore binding site on apophytochrome is best tailored to phytochromomobilin, apophytochrome will accommodate the two analogs with modified D-rings, phycocyanobilin and phycoerythrobilin. The phycocyanobilin-apophytochrome adduct is photoactive and undergoes a light-induced protein conformational change similar to the native holoprotein. By contrast, the phycoerythrobilin adducts is locked into a photochem. inactive protein conformation that is similar to the red light-absorbing Pr form of phytochrome. Thus, photoconversion from Pr to Pfr, the far red light-absorbing form of phytochrome, involves the photoisomerization of the C15 double bond. Rational design of chromophore analogs whose insertion into apophytochrome should elicit profound changes in light-mediated plant growth and development.

L8 ANSWER 9 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 7
ACCESSION NUMBER: 1992:546832 CAPLUS
DOCUMENT NUMBER: 117:146832
TITLE: Phytochrome assembly. The structure and biological activity of 2(R),3(E)-phytochromobilin derived from phycobiliproteins
AUTHOR(S): Cornejo, Juan; Beale, Samuel I.; Terry, Matthew J.; **Lagarias, J. Clark**
CORPORATE SOURCE: Div. Biol. Med., Brown Univ., Providence, RI, 02912, USA
SOURCE: J. Biol. Chem. (1992), 267(21), 14790-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The unicellular rhodophyte Porphyridium cruentum and the filamentous cyanobacterium Calothrix sp. PCC 7601 contain phycobiliproteins that have covalently bound phycobilin chromophores. Overnight incubation of solvent-extd. cells at 40.degree. with methanol liberates free phycobilins that are derived from the protein-bound bilins by methanolytic cleavage of the thioether linkages between bilin and **apoprotein**. Two of the free bilins were identified as 3(E)-phycocyanobilin and 3(E)-phycoerythrobilin by comparative spectrophotometry and HPLC. Methanolysis also yields a third bilin free acid whose absorption and 1H NMR spectra support the assignment of the 3(E)-phytochromobilin structure. This novel bilin is the major pigment isolated from cells that are pre-extd. with acetone-contg. solvents. Since phytochrome- or phytochromobilin-contg. proteins are not present in either organism, the 3(E)-phytochromobilin must arise by oxidn. of phycobilin

chromophores. This pigment is not obtained by similar treatment of a cyanobacterium and a rhodophyte that lack phycoerythrin. Therefore, 3(E)-phytochromobilin appears to be derived from phycoerythrobilin-contg. proteins. Comparative CD spectroscopy of 3(E)-phytochromobilin and 3(E)-phycocyanobilin suggests that the two bilins share the R stereochem. at the 2-position in the reduced pyrrole ring. Incubation of 2(R),3(E)-phytochromobilin with recombinant oat apophytochrome yields a covalent bilin adduct that is photoactive and spectrally indistinguishable from native oat phytochrome isolated from etiolated seedlings. Thus, phycobiliprotein-derived 2(R),3(E)-phytochromobilin is a biol. active phytochrome chromophore precursor.

L8 ANSWER 10 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 8
ACCESSION NUMBER: 1992:52545 CAPLUS
DOCUMENT NUMBER: 116:52545
TITLE: Expression and assembly of spectrally active recombinant holophytochrome
AUTHOR(S): Wahleithner, Jill A.; Li, Liming; **Lagarias, J. Clark**
CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1991), 88(23), 10387-91
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To develop an in vitro phytochrome assembly system, the authors expressed an oat phytochrome cDNA in both the yeast Saccharomyces cerevisiae and the bacterium Escherichia coli. Anal. of sol. protein exts. showed that the recombinant apophytochromes were full-length and capable of covalently attaching the phytochrome chromophore analog phycocyanobilin. Difference spectra indicated that in vitro-assembled holophytochrome species were photoreversible; however, max. and min. difference absorption values were blue-shifted relative to those of the native photoreceptor. Exts. contg. the recombinant apophytochromes were also incubated with phytochromobilin, the natural chromophore synthesized from biliverdin by cucumber etioplast prepns. In these expts., the difference spectrum obtained was identical to that of native oat holophytochrome. These results suggest that the recombinant apophytochromes adopt a structure similar to that of the **apoprotein** biosynthesized in vivo. ELISAs were used to quantitate phytochrome expression levels in both yeast and E. coli exts. These measurements show that 62-75% of the phytochrome **apoprotein** in the sol. protein ext. was competent to assemble with bilins to form spectrally active holophytochrome.

L8 ANSWER 11 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 9

ACCESSION NUMBER: 1989:529120 CAPLUS
DOCUMENT NUMBER: 111:129120
TITLE: Formation of a photoreversible phycocyanobilin-apophytochrome adduct in vitro
AUTHOR(S): Elich, Tedd D.; **Lagarias, J. Clark**
CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: J. Biol. Chem. (1989), 264(22), 12902-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Avena seedlings grown in the presence of the plant tetrapyrrole synthesis inhibitor 4-amino-5-hexynoic acid contains <10% of the spectrally detectable phytochrome levels found in untreated seedlings, but continue to accumulate phytochrome **apoprotein** . Using such tetrapyrrole-deficient seedlings, it was previously reported that phycocyanobilin, the cleaved prosthetic group of C-phycocyanin, can be incorporated into phytochrome in vivo to yield spectrally active holoprotein. Addn. of phycocyanobilin to sol. exts. of inhibitor-treated seedlings results in a rapid increase in spectrally active phytochrome holoprotein. The newly formed photoactive species displays a blue-shifted absorbance difference spectrum similar to that obsd. in the previous in vivo studies. The increase in spectral activity is consistent with conversion of all of the preexisting phytochrome **apoprotein** to functionally active holoprotein. The formation of a covalent phycocyanobilin-apophytochrome adduct is shown by an increase in Zn2+-dependent bilin fluorescence of the phytochrome polypeptide. A photoreversible, covalent adduct with a similar optical spectrum also forms when immunopurified apophytochrome is incubated with phycocyanobilin. ATP, reduced pyridine nucleotides, or other cofactors are not required for adduct formation. When biliverdin IX.alpha. is substituted for phycocyanobilin, no spectrally active covalent adduct is produced. These results indicate that an A-ring ethylidene-contg. bilatriene is required for post-translational covalent attachment of bilin to apophytochrome and that apophytochrome may be the bilin C-S lyase which catalyzes bilin attachment.

L8 ANSWER 12 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 10
ACCESSION NUMBER: 1989:530835 CAPLUS
DOCUMENT NUMBER: 111:130835
TITLE: Self-assembly of synthetic phytochrome holoprotein in vitro
AUTHOR(S): **Lagarias, J. Clark**; Lagarias, Donna M.
CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1989), 86(15), 5778-80

CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The phytochrome holoprotein of plants requires a covalently bound linear tetrapyrrole (bilin) prosthetic group for its photoreceptor function. The synthetic phytochrome **apoprotein** prepd. by transcription and translation of an Avena phytochrome cDNA construct combines in vitro with phycocyanobilin, an analog of the natural chromophore, to produce a photoactive holoprotein. Thus, holoprotein assembly is an autocatalytic process.

L8 ANSWER 13 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 11
ACCESSION NUMBER: 1987:493589 CAPLUS
DOCUMENT NUMBER: 107:93589
TITLE: Phytochrome chromophore biosynthesis. Both 5-aminolevulinic acid and biliverdin overcome inhibition by gabaculine in etiolated Avena sativa L. seedlings
AUTHOR(S): Elich, Tedd D.; **Lagarias, J. Clark**
CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: Plant Physiol. (1987), 84(2), 304-10
CODEN: PLPHAY; ISSN: 0032-0889
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Etiolated A sativa seedlings grown in the presence of gabaculine (5-amino-1,3-cyclohexadienylcarboxylic acid) contained reduced levels of phytochrome as shown by spectrophotometric and immunochem. assays. Photochromic phytochrome levels in gabaculine-grown plants were estd. to be 20% of control plants, while immunoblot anal. showed that the phytochrome protein moiety was present at approx. 50% of control levels. Gabaculine-grown seedlings administered either 5-aminolevulinic acid or biliverdin exhibited a rapid increase of spectrophotometrically detectable phytochrome. Phytochrome concns. estd. immunochem. did not similarly increase throughout treatment with either compd. Similar expts. with 5-amino[4-14C]levulinic acid showed radiolabeling of phytochrome with kinetics that paralleled the spectrally detected increase. These results are consistent with the intermediacy of both 5-aminolevulinic acid and biliverdin in the biosynthetic pathway of the phytochrome chromophore and the lack of coordinate regulation of chromophore and **apoprotein** synthesis in Avena seedlings.

L8 ANSWER 14 OF 14 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 12
ACCESSION NUMBER: 1982:577246 CAPLUS
DOCUMENT NUMBER: 97:177246
TITLE: Bile pigment-protein interactions. Coupled oxidation of cytochrome c
AUTHOR(S): **Lagarias, J. Clark**

CORPORATE SOURCE: Dep. Biochem. Biophys., Univ. California, Davis, CA, 95616, USA
SOURCE: Biochemistry (1982), 21(23), 5962-7
CODEN: BICHAW; ISSN: 0006-2960
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The selective chem. modification of the heme prosthetic group of horse heart cytochrome c is facilitated by utilizing coupled oxidn. with O2 and ascorbate in the presence of large amts. of pyridine. Comparison of the absorption spectra of this chem. modified cytochrome c species in 2 different solvents (aq. pyridine and CO-satd. 6M guanidine-HCl) with those of 2 model compds. [bis(pyridine)(2,3,7,8,12,13,17,18-octaethyl-5-oxaporphyrinato)iron(II) tetrafluoroborate salt and (pyridine)carbonyl(2,3,7,8,12,13,17,18-octaethyl-5-oxaporphyrinato)iron(II) tetrafluoroborate salt] showed that coupled oxidn. of cytochrome c afforded a new protein with a covalently bound Fe(II) oxaporphyrin prosthetic group. Amino acid anal. of this protein-bound Fe(II) oxaporphyrin species revealed that only limited modification of the primary structure of the **apoprotein** occurred during the coupled oxidn. of cytochrome c. This protein-bound Fe(II) oxaporphyrin species was also interconvertible to a protein-bound bilatriene species under hydrolytic conditions. The synthetic utility of the coupled oxidn. of cytochrome c for the prepn. of chromoproteins which possess covalently bound Fe(II) oxaporphyrin and bilatriene prosthetic groups is considered.

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